In Science the credit goes to the man who convinces the world, not to the man to whom the idea first occurred.

- Sir William Osler (c. 1849-1919)

University of Alberta

Examination of the Role of the Phosphatidylinositol (PI) Cycle in Bipolar and Unipolar Depression

by

Brent Matthew McGrath ©

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Abstract

Mood disorders, including bipolar disorder and unipolar major depression, are among the most prevalent and debilitating of the psychiatric illnesses. Of the symptoms experienced, depression is the most common and life threatening, particularly due to the increased risk of suicide during these episodes. The application of magnetic resonance spectroscopy meant that changes in specific brain metabolite concentrations could be measured during different symptom states and following treatment and recovery.

This thesis aimed to better characterize the involvement of *myo*-inositol in the treatment and pathophysiology of bipolar and unipolar depression. Male Sprague-Dawley rats were treated with a number of mood stabilizers and antidepressants to determine the effect, if any, on *myo*-inositol concentrations in several brain regions, including the prefrontal, temporal and occipital cortices as well as the hippocampus. Acute administration of dextro-amphetamine was also evaluated in terms of its effects on *myo*-inositol concentrations in the brains of healthy human volunteers and rats. Finally, *myo*-inositol concentrations in the dorsomedial prefrontal cortex of bipolar type II depressed and unipolar depressed patients were assessed relative to age- and sex-matched healthy controls.

Results from the present set of experiments are: [1] *Myo*-inositol concentrations did not differ between bipolar type II depressed patients, unipolar depressed

patients and healthy controls, although there was a numeric increase in the bipolar group. [2] An acute dose of dextro-amphetamine did not alter *myo*-inositol concentrations in the brains of healthy volunteers or rats in a manner consistent with clinical changes observed in patients with bipolar mania. [3] Long-term (2 and 4 week) but not short-term (1 week) treatment with lithium reduces regional rat brain *myo*-inositol concentrations. [4] Contrary to the effects of lithium, other mood stabilizers did not affect regional *myo*-inositol concentrations in the brains of rats. [5] Similarly, antidepressants belonging to three distinct chemical classes did not change regional *myo*-inositol concentrations in the brains of rats.

Myo-inositol concentration changes in humans and rats may be specific to treatment with lithium. Other medications may act on the PI-cycle, but not by altering *myo*-inositol concentration. Also, it is unlikely that alterations in *myo*-inositol are a marker for mood disorders.

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Dedication

This thesis is for all those who dare to ask, and particularly for those who strive to answer.

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List of Abbreviations

α_{1A}	adrenergic 1A
α_{1B}	adrenergic 1B
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BCE	before common era
b.i.d	twice a day
B_0	static magnetic field
BPD	bipolar disorder
BPD-I	bipolar disorder type I
BPD-II	bipolar disorder type II
cAMP	adenylyl cyclase
Ca^{2+}	calcium
cm	centimeter
CMP-PA	cytidine monophosphorylphosphatidate
cMRI	conventional magnetic resonance imaging
CNS	central nervous system
Cr	creatine
Cr-PCr	creatine+phosphocreatine
CSF	cerebrospinal fluid
d	doublet
dd	doublet of doublets
D_1	dopamine 1
D_2O	deuterium oxide
DAG	1,2-diacylglycerol
DLPFC	dorsolateral prefrontal cortex
DMPFC	dorsomedial prefrontal cortex
DNA	deoxyribonucleic acid
DRESS	depth-resolved surface spectroscopy

DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th edition test-revised
DSS	2,2-dimethyl-2-silapentane-5-sulfonate
fMRI	functional magnetic resonance imaging
g	gram
GABA	γ-aminobutyric acid
GAF	global assessment of functioning
G-6-P	glucose-6-phosphate
Glx	glutamine+glutamine
GM	grey matter
GSK-3	glycogen synthase kinase-3
G_q	guanine nucleotide-binding protein
H_2O	water
HAM-D	Hamilton depression rating scale
HC	healthy control(s)
HCN	^{1}H,^{13}C,^{15}N
HIV	human immunodeficiency virus
HSD	Tukey's honestly significant difference test
HV	healthy volunteers
Hz	hertz
IMPase	inositol monophosphatase
Ins[1,4,5]P_3	inositol 1,4,5-trisphosphate or IP_3
IP	intraperitoneal
IP_1	inositol-1-monophosphate
ISIS	image-selected in vivo spectroscopy
K	kelvin
L-COSY	two-dimensional localized chemical shift correlated spectroscopy
LCModel	linear combination of metabolite basis spectra
Li^+	lithium
m	multiplet
M	molar

MADRS	Montgomery-Asberg depression rating scale
mM	millimolar
M_1	muscarinic cholinergic 1
M_3	muscarinic cholinergic 3
MAOI	monoamine oxidase inhibitor
$mGlu_1$	metabotropic glutaminergic 1
$mGlu_5$	metabotropic glutaminergic 5
M	metabolite
MCB	metabolite concentration in brain
MHz	megahertz
m-Ino	*myo*-inositol
ml	milliliter
mm	millimeter
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MSB	metabolite signal from brain
msec	milliseconds
NAA	*N*-acetylaspartate
NMR	nuclear magnetic resonance
PCr	phosphocreatine
PDE	phosphodiesters
PEPSI	photon echo-planar spectroscopic imaging
PET	positron emission tomography
PI	phosphatidylinositol
PI-PLC	phosphatidylinositol-phospholipase C
PIP	phosphatidylinositol phosphate
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C
PLC	phospholipase C
PME	phosphomonoester

PO	prolyl oligopeptidase
ppm	parts per million
PRESS	point-resolved spectroscopy
PWC	pure water concentration
q	quartet
rf	radio frequency
RNA	ribonucleic acid
s	singlet
SCID	structured clinical interview for DSM-IV axis I disorders
sec	second
SEM	standard error of the mean
SF	scaling factor
S/N	signal to noise
SNRI	serotonin and norepinephrine reuptake inhibitor
SPECT	single photon emission computed tomography
SSRI	selective serotonin reuptake inhibitor
STEAM	stimulated echo acquisition mode
t	triplet
T	tesla
T_1	longitudinal (or spin lattice) relaxation time
T_2	transverse (or spin spin) relaxation time
TCA	tricyclic antidepressant
TE	echo time
TR	repetition time
UPD	unipolar major depressive disorder
VAS	visual analogue scale
W	water
WCB	water concentration in brain
WHO	World Health Organization
WM	white matter
WSB	water signal from brain

YMRS	Young mania rating scale
^{o}C	degrees Celsius
μmol	micromole
γ	gyro-magnetic ratio
ω_0	Larmor (precessional) frequency
1D	one dimensional
^{1}H	hydrogen-1 or proton
5-HT	5-hydroxytryptamine or serotonin
5-HT_{1A}	serotonin 1A
5-HT_{1C}	serotonin 1C
5-HT_2	serotonin 2
^{7}Li	lithium-7
^{13}C	carbon-13
^{31}P	phosphorus-31

Chapter 1. Introduction

(Parts of this chapter have been published in *Current Psychiatry Reviews*, *Bipolar Disorders*, *Human Psychopharmacology*, *Canadian Journal of Psychiatry* and the *McGill Journal of Medicine* – see Silverstone et al. 2005a; Silverstone et al. 2005b; Kim et al. 2005; McGrath et al. 2004; McGrath 2004)

1.1 Mood Disorders

Mood is a pervasive and sustained emotion that colours the person's perception of the world (Sadock and Sadock 2003). When referring to mood, some of the common adjectives used often include depressed, despairing, irritable, anxious, angry, euphoric, guilty, hopeless, frightened and manic. A person's mood may be labile, fluctuating or alternating rapidly between extremes (Sadock and Sadock 2003). While healthy individuals experience all mood states, they feel largely in control of their moods. On the contrary, among patients with mood disorders, the sense of control is lost, and one's mood is often incongruent with the context in which the individuals find themselves. This is cause for a subjective experience of great distress.

Historically, mood (or affective) disorders were classified in a number of ways, with distinctions made between endogenous and reactive episodes, psychotic and neurotic symptomatology and episodes arising *de novo* (primary) in contrast to those arising in the context of another disorder (secondary) (Kendell 1976; Farmer and McGuffin 1989). In modern nosological classification, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), there are two broad categories of mood disorders, unipolar major depressive disorder (UPD) and bipolar disorder (BPD), formerly manic-depressive illness (American Psychiatric Association 2000). Put in context, in its Global Burden of Disease study, the World Health Organization (WHO) noted that UPD and BPD are, respectively, the first and sixth ranked causes of years lived with disability among people in developed nations (Murray and Lopez 1996; Ustun and Chisholm

2001). Furthermore, the WHO predicts that by 2020 UPD will be the second largest (behind ischemic heart disease) cause of global disease burden (Murray and Lopez 1996). This highlights the importance of developing a more thorough understanding of the pathophysiology and aetiology of these two common, debilitating and costly disorders. Primary mood disorders (including BPD and UPD) are generally classified according to both the nature and severity of symptoms experienced during each episode, and by the overall course of the illness (Gelder et al. 2001).

1.1.1 Unipolar Major Depression

Unlike the normal shifts in mood that most people experience, the symptoms of depression are more extreme and frequently incapacitating. Hippocrates was one of the first to describe depression, referring to it as melancholia and attributing it to an excess of black bile, in accordance with the four humours theory of illness. In the Hippocratic writings, melancholia was characterized by an aversion to food, despondency, sleeplessness, irritability and restlessness (Jouanna 1999; Jacquart 2004).

Today, many of Hippocrates's early descriptions remain central to the diagnosis of depression. The modern list of symptoms includes a persistent sad mood, loss of interest or pleasure in activities that were once enjoyed, significant change in appetite or body weight, difficulty sleeping or oversleeping, physical slowing or agitation, loss of energy, feelings of worthlessness or inappropriate guilt, difficulty thinking or concentrating, and recurrent thoughts of death or suicide (American Psychiatric Association 2000). To be diagnosed with depression, an individual must be experiencing at least five of these symptoms every day over a two-week period (Sadock and Sadock 2003). Using this definition, and depending on the source, 5-25% of people will experience an episode of major depression, which accounts for approximately 50% of all psychiatric hospitalizations (Angst 1995). In Canada, the lifetime prevalence rate has been reported to be about 8.5% (Spaner et al. 1994). The mean age of onset for a major depressive episode is about 40 years. Unlike BPD, UPD is twice as common in women (Angst 1995) and unipolar depressive episodes typically last longer than do episodes of bipolar depression. Common psychiatric comorbidities include substance abuse and anxiety disorders (Spaner et al. 1994).

Theories regarding the pathophysiology of UPD have closely mirrored the mechanisms by which it is supposed that the antidepressant medications offer their symptomatic relief. Interestingly, the original discovery of the effectiveness in depression of the earliest antidepressants, iproniazid (a monoamine oxidase inhibitor) and imipramine (a tricyclic compound), was largely serendipitious (Kuhn 1957; Kuhn 1958; Deverteuil and Lehmann 1958; Lehmann et al. 1958). Both of these compounds were shown to increase the bioavailability of monoamines in the synaptic cleft. Soon after their discovery, the first monoamine theory of depression was proposed (Schildkraut 1965). In this theory, Schildkraut (1965) stated that; "some, if not all, depressions are associated with an absolute or relative deficiency of catecholamines, particulary noradrenaline, at functionally important adrenergic receptor sites in the brain". He also proposed one of the earliest biochemical theories of mania, where "elation may be associated with an excess of such amines" (Schildkraut 1965). A few years after the noradrenaline theory of depression, serotonin was suggested as being involved in depressive pathophysiology (Coppen 1967; Oxenkrug and Lapin 1971), based largely on the same evidence. The involvment of serotonin received renewed support with the introduction and subsequent widespread use of the selective serotonin reuptake inhibitors (SSRI) in the 1980's. Dopamine may also play a role in depressive physiology, with several studies reporting increased dopamine receptor gene expression (Lammers et al. 2000) and increased dopamine receptor numbers (Dziedzicka-Wasylewska et al. 1997; Ainsworth et al. 1998) in animals treated with antidepressants. Animal studies have also supported the involvement of the monoamines in stress and emotional behaviour (Cooper et al. 2003; Dziedzicka-Wasylewska et al. 1997). Although much of the research has been supportive of an enhanced brain availability of one or more of the monoamines for maintaining an antidepressant response, there is not substantial evidence supporting one over the others. Moreover, some of the effective antidepressants influence one, several or none of the monoamine neurotransmitters and the time course between alterations in monoamine levels at the synapse and the improvement in symptomatology is often separated by several weeks (Blier 2001). It remains unclear as to whether or not a disruption in monoamine systems is responsible for major depressive disorder (Slattery et al. 2004). As a result, research moved beyond the monoamines, investigating other neurotransmitters including γ-aminobutyric acid (GABA) (Petty 1995; Lloyd et al. 1985) and glutamate (Papp and Moryl 1994), and more recently, neuroactive peptides and steroids including substance P and cholecystokinin (Steinberg et al. 2001) and growth factors including brain derived neurotropic factor (Karege et al. 2002; Chen et al. 2001). Also of interest are the second messenger systems, including the phosphatidylinositol cycle (PI-cycle), which is linked to a number of the receptors that are effectors for the monoamine neurotransmitters.

1.1.2 Bipolar Disorder

Bipolar disorder is a multifactorial psychiatric illness (Bezchlibnyk and Young 2002; Alda 2004), occurring in several forms and degrees of severity (Goodwin and Jamison 1990). Originally described in the first century before the common era (BCE) by Soranus (Marneros and Angst 2000), and defined by Aretaeus (Adams 1856), the shift between depressive stupor, euthymic serenity and manic excitement generally characterize the existence of the bipolar patient. These states, in turn, consist of disturbances in mood, cognition, perception and behaviour (McElroy et al. 1996; Bearden et al. 2001). In contrast to depression, mania is marked by periods of abnormally and persistently elevated mood or irritability often accompanied by overly inflated self-esteem, a decreased need for sleep, increased talkativeness, racing thoughts, distractibility, increased goal-directed activity or physical agitation, and excessive involvement with pleasurable activities that have a high potential for risky consequences (American Psychiatric Association 2000). An individual must be experiencing three or more of these symptoms for at least a one-week period for a diagnosis of mania and for a four-day period for a diagnosis of hypomania (Sadock and Sadock 2003). In recent diagnostic criteria, the type of BPD has been distinguished by the presence of either mania or hypomania. Bipolar disorder type I (BPD-I) is characterized by a cyclic succession of manic or mixed states with episodes of depression (Thomas 2004). The concept of bipolarity was further extended by Dunner and associates (1976) to include bipolar disorder type II (BPD-II), characterized by an unstable course of alternating depression and hypomania (Thomas 2004). Support for the clinical and diagnostic distinction of BPD-II from both BPD-I and UPD stemmed from differences in clinical presentation, as well as differences in drug response (Dunner et al. 1976; Ayuso-Gutierrez and Ramos-Brieva 1982; Simpson and DePaulo 1991; Simpson et al. 2002).

Medical consultation or first hospitalization for BPD generally occurs between the ages of 25 and 30 (Marneros and Brieger 2002), usually as a result of manic symptoms (Tomb 1999). The mean age of onset of bipolar mania has been reported to be at about 20 years of age (Fogarty et al. 1994). However, sub-acute symptom onset is believed to occur many years preceding this (Goodwin and Jamison 1990), with depressive symptoms most frequently reported at first episode. The course of BPD is usually a chronic, cycling one. Classically, the lifetime prevalence of BPD was estimated to be about 1%, equal among men and women (Regier et al. 1988); however, current evidence lends more support to an estimate of about 5% (Angst 1998). The discrepancy can, in part, be attributed to a shift in the conceptualization of the disorder, from a unipolar-bipolar dichotomy to that of a bipolar spectrum (Akiskal and Pinto 1999; Hirschfeld 2004) and in methodological differences in the inclusion/exclusion criteria; namely, whether or not individuals with BPD-II were included in the prevalence studies. Several studies have shown that BPD-II is 4 – 5 times more prevalent that BPD-I (Weissman et al. 1990; Kessler et al. 1994; Szadoczky et al. 1998; Akiskal et al. 2000). A recent Canadian study of 36,984 respondents puts the prevalence somewhere in between, at about 2.2% (Schaffer et al. 2006). In this study, Ontario had the lowest prevalence (1.9%) while British Columbia had the highest (2.9%), with BPD being more common among individuals in the low income demographic (total yearly household income less than $15,000) (Schaffer et al. 2006). Common comorbidities include substance abuse and anxiety disorders (Fogarty et al. 1994). To date, the pathophysiological underpinnings underlying these extreme swings in mood are not understood.

Despite their frequency and severity, to date we have only limited data regarding the neurobiology underlying both BPD and UPD. Moreover, our limited knowledge into the workings of a healthy brain have only complicated efforts to understand the pathophysiological changes in patients with mood disorders; an understanding that requires linking the complex behavioral manifestations, functional deficits and the cyclic nature of the disorder with its neurochemical, neurobiological, and genetic underpinnings (Berns and Nemeroff 2003). Several studies have suggested that there are differences between BPD patients and healthy controls in many areas, as well as some differences between BPD patients and patients with UPD. Data from family, twin and adoption studies indicate a genetic component, or a genetically transmitted vulnerability (Kelsoe 2003). While the lifetime prevalence of BPD-I is approximately 1% in the general population, familial studies have pointed to a prevalence 10- to 20-times greater among first-degree relatives of bipolar probands (Merikangas et al. 2002), with concordance rates as high as 60% reported in monozygotic twins (Kendler et al. 1993). Moreover, families of bipolar probands also have a higher prevalence for other mood disorders and symptoms (Gershon et al. 1982), possibly indicative of a shared genetic basis among diagnostically distinct affective phenotypes. It is unlikely that this genetic vulnerability is due to any single major locus, but may involve a more complex, polygenic mode of transmission. In terms of the two subtypes of BPD, genetic studies suggest that there may also be differences between BPD-I and BPD-II patients. In support of this, studies have reported that relatives of BPD-II probands are more likely to suffer from BPD-II or UPD than they are from BPD-I (Gershon et al. 1982; Heun and Maier 1993). Bipolar type II disorder may even breed true in some families (Benazzi 2004). If this is indeed the case, it is possible that with the application of diverse methodologies, the pathophysiology of any such differences may be better understood.

1.1.3 Treatment of Mood Disorders

With regard to the biological treatment of mood disorders, the expansion in the array of available pharmacological medication has provided effective treatment options for clinicians and greater symptomatic relief for patients.

1.1.3.1 Mood Stabilizers

To date, the mainstay of treatment for patients with BPD is the monovalent cation, lithium (Fieve 1999; Yatham et al. 2005). Lithium's utility and efficacy as a psychoactive agent – particularly in the treatment of the manic symptoms of BPD – was serendipitously re-discovered[1] by John Cade, an Australian psychopharmacologist, in 1949 (Cade 1949). It is now well established that lithium is effective in both the acute treatment of mania and the long-term prophylaxis of manic and depressive episodes (Post et al. 2000). However, findings to date have reported that lithium is only effective in between 50% and 70% of BPD patients (Davis et al. 1999; Kusumaker et al. 1997). To improve on this, other agents with mood-stabilizing properties have been introduced. Among these, the anticonvulsants sodium valproate, a propylpentanoate, and carbamazepine, a carboxamide, have the most supportive evidence and are currently the most commonly prescribed. Both sodium valproate and carbamazepine have been reported to be as effective as lithium in the acute treatment of mania (Bowden et al. 1994; Macritchie et al. 2003; Kusumakar et al. 1997). These agents do not share lithium's clinical utility for the treatment of bipolar depression. Together, all three agents share a common profile in the treatment of BPD. Namely, all three agents are reportedly more effective in treating acute mania than acute depression (Calabrese 2000), and all have reported utility in long-term prophylaxis (Post et al. 2000).

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[1] Lithium was originally discovered by Johann August Arfvedson in 1817. In 1880 it was introduced into the field of psychiatry, by John Aulde and Carl Lange, for the prophylactic treatment of depression. Shortly after 1880, cases of lethal lithium toxicity resulted in widespread discontinuation of lithium in medicine, only to be re-introduced following publication of the findings of John Cade in 1949.

Recently, the phenyltriazine anticonvulsant, lamotrigine, was found to have efficacy in the treatment of BPD-I, particularly in the depressive phase of the illness (Calabrese et al. 2003; Calabrese et al. 1999; Frye et al. 2000). This is particularly important given that it is the depressive symptoms that are most responsible for morbidity and mortality in patients with BPD (Baldassano et al. 2003). However, there is no demonstrated efficacy for lamotrigine in the treatment of acute manic symptoms (Goldsmith et al. 2004). Lamotrigine's mechanism of action in BPD is not well understood. It has been shown to decrease glutamate concentrations, inhibit calcium and sodium channels – the likely mechanism by which it inhibits epileptic seizures – and to down-regulate cortical serotonergic 1A (5-HT$_{1A}$) receptor-mediated adenyl cyclase activity (Paraskevas et al. 2006; Goldsmith et al. 2003). No clear evidence exists to support the use of other anticonvulsant medications (i.e. vigabatrin and topiramate) in the treatment of BPD (Gajwani et al. 2005).

Conventionally known for the their calming and sedating effects, the use of typical antipsychotics in the treatment of BPD met with limited success. According to Tohen and Tollefson (2000), the side-effect profile and the high risk of inducing depressive symptoms and of increasing cyclicity made the typical antipsychotics a less than ideal treatment choice for BPD. However, the introduction of the atypical antipsychotics sparked a renewed interest in the use of this class of medication in patients with BPD, particularly in those patients who are non-responsive to traditional mood-stabilizing agents. Moreover, the improved profile of the atypical antipsychotics has provided a new avenue of treatment options for BPD. Among these agents, the thienobenzodiazepine, olanzapine, has offered the most promise; however, more research is needed to determine the range of symptom coverage for these new generation antipsychotics, both alone and in combination with other mood-stabilizing agents.

Evidence suggests that olanzapine can delay relapse of both manic and depressive symptoms at least as effectively as sodium valproate and lithium (Tohen et al. 2003; Tohen et al. 2005).

Akin to this development has been a change in the definition of what constitutes a mood-stabilizing agent. Early attempts at defining the ideal mood-stabilizing agent focused on the agent's ability to treat at least one principal symptom of the illness (mania or depression) without worsening any other aspect, while still being safe and well tolerated (Akiskal 2002). While not entirely abandoned, Kasper (2002) makes the point that successful symptom treatment and prevention in BPD is not easily attainable with the use of only one medication, indicating a need for a "multi-pronged" approach. According to Keck and colleagues (2002), this can be explained in terms of a change in the definition of the properties that characterize mood-stabilizing medication as a result of variation in both the properties of specific medications and the clinical characteristics of the illness. It is important to note that the majority of the treatment studies have assessed the effectiveness of these medications in patients with BPD-I, and that their effectiveness in BPD-II has been largely inferred. This dearth of knowledge is unfortunate given the relatively higher prevalence of this subtype of BPD. For a more extensive discussion of the clinical evidence surrounding the effectiveness, safety and tolerability of the various mood stabilizing agents available, interested readers are referred to the excellent review by Yatham and associates (2005).

1.1.3.2 Antidepressants

Early observations that reserpine, which depletes brain monoamines and decreases monoamine reuptake and that amphetamine, which increases monoamine release and decreases monoamine reuptake, pointed toward the involvement of serotonin, norepinephrine and dopamine in mood regulation. Serotonin (or 5-hydroxytryptamine, 5-HT), an indolealkylamine, is found in several cell types, with only about 1 – 2% found in nervous tissue (Cooper et al. 2003). Serotonin does not cross the blood-brain barrier and must be synthesized from dietary tryptophan in brain. Serotonergic neurons originate from raphe nuclei of the brain stem and project to cortical and subcortical areas throughout the central nervous system, including the frontal cortex, hypothalamus and amygdala (Gorman and Kent 1999). Mood is purported to be influenced by those projections to the frontal cortex (Gorman and Kent 1999). Norepinephrine, a catecholamine, was first identified as the neurotransmitter for sympathetic adrenergic neurons of the peripheral nervous system (Crandell 1963). Norepinephrine is synthesized from tyrosine. In brain, the majority of noradrenergic neurons project from the locus ceruleus to almost all other brain regions (Nestler et al. 2001). Recent evidence suggests that noradrenergic neurons of the locus ceruleus have several functions, including a role in learning and memory, attention, reinforcement and the brain's general responsiveness to external events (Aston-Jones et al. 1999; Cooper et al. 2003). Dopamine is the chemical precursor to norepinephrine in the brain, and like norepinephrine is synthesized from tyrosine. In brain, there are four primary dopamine pathways that have been studied in the context of psychiatric disorders, with neuronal projections originating from the substantia nigra pars compacta, the ventral tegmental area and the arcuate nucleus of the hypothalamus (Cooper et al. 2003). Projections from the substantia nigra pars compacta run to regions of the striatum, while projections from the ventral tegmental area lead to limbic areas including the nucleus accumbens and areas of the prefrontal cortex and cingulated cortex. These two pathways form the mesocorticolimbic dopamine system. The third pathway – the tuberoinfundibular dopamine system – projects from the arcuate nucleus to the median eminence area of the hypothalamus and is primarily involved in hormonal control vis-a-vis pituitary prolactin release. The functions of these pathways are diverse and range from control of voluntary motor function, effects on learning and memory, to regulation of lactation (Nestler et al. 2001).

Initial work with isoniazid, a drug used to treat tuberculous, led to the synthesis of iproniazid. When given to treat tuberculous, iproniazid was observed to cause psychostimulation (Pleasure 1954). Iproniazid was also found to inhibit monoamine oxidase (Dick 1959) and to reverse the sedative effects of reserpine (Saunders et al. 1959). These unexpected actions lead to iproniazid's use as the first antidepressant (Pletscher 1991), and as the first member of the monoamine oxidase inhibitor (MAOI) class. This class of antidepressant is purported to exert its therapeutic effects by inhibiting the breakdown of serotonin and norephinephrine by MAO, leading to sustained neurotransmitter action via an accumlation in the synaptic cleft. Another class of antidepressants, the tricyclic antidepressants (TCA), were also introduced around the same time as the MAOIs, but were reported to be effective by inhibiting the active reuptake of monoamines into the presynaptic terminal, leading to an effect on post-synaptic receptors that is similar to that induced by the MAOIs. Over a period of five decades, several classes of antidepressants have been developed that affect one, two or all of these neurotransmitters.

Based on the early success of the TCA and MAOI antidepressants in the early 1980's researchers introduced zimelidine, which belonged to a new class of antidepressant medication (Montgomery et al. 1981). This class became known as the SSRIs, due to their selective inhibition of serotonin reuptake by pre-synaptic terminals, with little or no effect on norepinephrine. Medications from this class of antidepressants have been among the most commonly prescribed for depression (Pincus et al. 1998) and include fluoxetine, paroxetine, sertraline and citalopram. This is due in part to their improved tolerability and side-effect profile, their safety in overdose, and their clinical utility in the spectrum of depressive disorders (Feighner 1999), particularly when compared to the TCA and MAOI antidepressants. That said, SSRIs are not effective for all patients, and studies have suggested that this class of antidepressant loses efficacy over time (reviewed by Byrne and Rothschild 1998).

In recent years several other types of antidepressant medications, which do not fit well into any of the previous three classes, have been utilized with increasing frequency. Included among these are venlafaxine, mirtazapine, trazodone and bupropion. Among these, venlafaxine has received much of the attention. Venlafaxine differs in its pharmacological profile from other antidepressants in that it acts on both serotonergic and noradrenergic neurons, inhibiting reuptake of both these neurotransmitters (Stahl et al. 2005) and it has an improved side-effect profile. As a result, venlafaxine has been refered to as a dual-acting agent, or a serotonin and norepinephrine reuptake inhibitor (SNRI). Evidence supports a link between the serotonergic and noradrenergic systems in depression (Cleare et al. 1997). Moreover, compounds, including some antidepressants, which affect serotonin, may have modulatory effects on norepinephrine and dopamine (Feighner 1999). This is suggestive of a single amine system dysfunction underlying the pathophysiology of depression (Tremblay and Blier 2006). In line with this idea, agents with dual action, and even newer agents with triple action (i.e. serotonin, norepinephrine and dopamine) (Popik et al. 2006), could potentiate events that lead to downstream changes, which may relate more to the core aetiology of depression (Feighner 1999). These changes may occur at the G-protein or second messenger level. Among these newer agents, venlafaxine has received the most clinical support to date, with evidence suggesting that it is more effective in the treatment of depression than the single acting SSRI fluoxetine (Dierick et al. 1996; Clerc et al. 1994). There is also evidence to suggest that venlafaxine may improve depressive symptoms in patients who were non-responsive to SSRIs (Thase et al. 2006), and that this agent has a more immediate onset of action, with symptom relief occurring in days rather than weeks (Szegedi 2006; Guelfi et al. 1995; Derivan et al. 1995).

It is important to note that the vast majority of studies assessing the effectiveness of antidepressant medications, of any class, have focused on their use in UPD. The use of antidepressant medication in bipolar depression has met with some controversy, due mainly to early evidence suggesting that antidepressant use can induce manic or hypomanic relapse (Prien et al. 1973; Lewis and Winokur 1982), increase episode frequency (Wehr and Goodwin 1979), and result in a poor long-term outcome (Maj et al. 2002). However, a recent review of the randomized controlled trails of antidepressant use in bipolar depression concluded that antidepressants might be of comparable efficacy in unipolar and bipolar depression, rates of switching to mania during the first 10 weeks of treatment are low, and that there is no strong evidence to suggest not using antidepressants (with the possible exception of TCAs) in patients with bipolar depression (Gijsman et al. 2004). Antidepressants may be particularly useful in BPD-II depressed patients (Amsterdam and Brunswick 2003), especially when given in conjunction with a mood stabilizer.

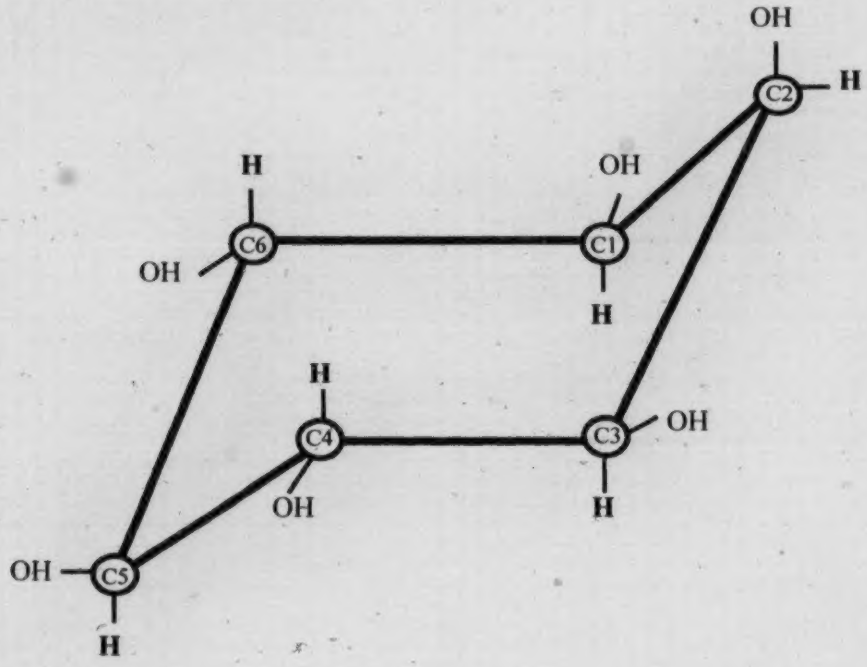
Thus, as noted by Keck and associates (2002), the most important goals of pharmacological treatment and management of mood disorders are the "prevention of mood episodes, eradication of interepisode subsyndromal symptoms and inhibition of the inherent cyclicity and mood lability". Key to this success is a unified understanding of both the neuropathophysiology of the condition as well as the mechanism(s) through which currently known effective medications offer relief. With this in mind, and while all the agents outlined above have some degree of mood-stabilizing properties, all belong to different chemical classes and have distinctly different structures. This has made it difficult to establish a unifying hypothesis regarding the means through which different medications act, and, by extension, the neuropathophysiology of these disorders. This has been particularly true for BPD, given that the medications known to be effective come from several different drug classes with diverse clinical applications.

1.2 *myo-Inositol*

A growing body of evidence suggests that alterations in brain *myo*-inositol may be associated with psychiatric disorders such as BPD (Gould et al. 2004), UPD (Coupland et al. 2005) and schizophrenia (Sharma et al. 1992), and to a lesser extent with neurological diseases such as Alzheimer's disease (Birchall and Chappell 1988) and hepatic encephalopathy (Tarasow et al. 2003). In order to understand how abnormalities in brain *myo*-inositol could be involved in such conditions, it is important to have some understanding of its role in the central nervous system (CNS).

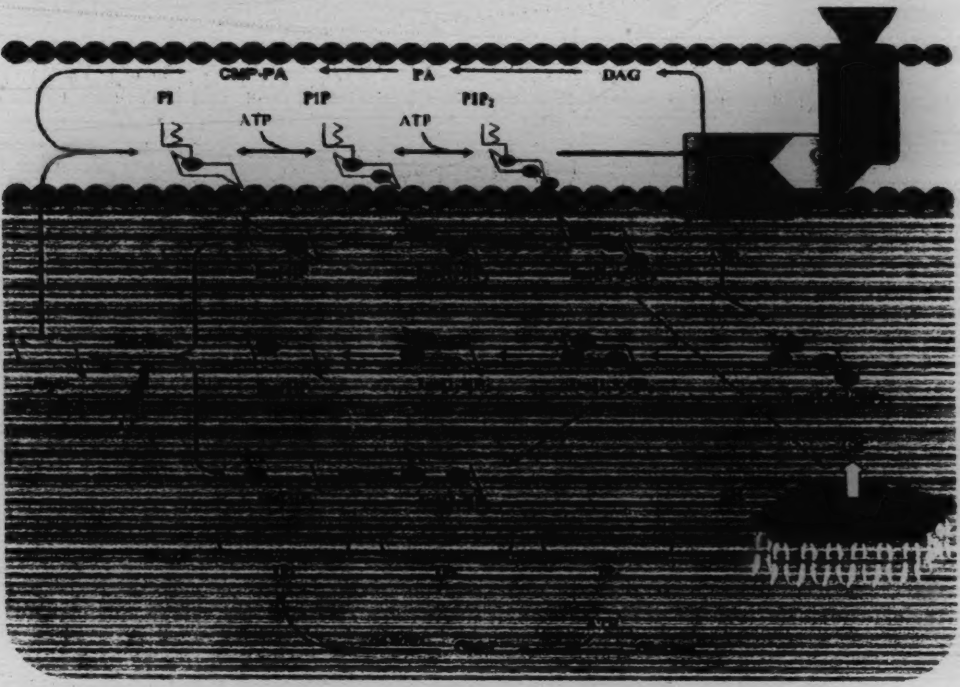
Inositol, a simple isomer of glucose, is an important dietary and cellular constituent (Colodny and Hoffman 1998). First isolated from muscle (Cantarow and Schepartz 1962; Doisy 1967), this ubiquitous, cyclic carbohydrate has a 6-carbon ring structure and can be found in any one of nine isomeric compositions, of which *myo*-inositol is the most abundant biologically active stereoisomer in the CNS (Ross 1991; Frey et al. 1998). *Myo*-inositol is unique among the inositol isomers, in that it has a single axial hydroxyl group at carbon 2 (Figure 1.1) (Vandal 1997), is an important growth factor for human cells (Holub 1982; Ross 1991), is an important non-nitrogenous CNS osmolyte (Thurston et al. 1989), is involved in transcriptional regulation and apoptosis (York et al. 2001; Fisher et al. 2002), and is a precursor in the PI-cycle second messenger system (Berridge and Irvine 1989; Berridge et al. 1989). It is the involvement in the PI-cycle that has received the most attention in relation to mood disorders.

Figure 1.1: The Structure of *myo*-Inositol. It has a symmetric ring structure with six carbons numbered sequentially.


OH
H
C2
H
OH
C6
C1
OH
H
H
C4
C3
OH
OH
H
OH
C5
H

In mammalian cells, a sustained supply of *myo*-inositol is required for the synthesis of membrane phospholipids, continuation of PI-cycle activity and the maintenance of intracellular free *myo*-inositol levels. It is synthesized in brain *de novo* from glucose-6-phosphate (G-6-P) (Holub 1982), first into inositol-1-monophosphate (IP_1) by IP_1 synthase, and subsequently into *myo*-inositol by inositol monophosphatase (IMPase) (Figure 1.2). The highest concentrations are found in brain, stomach, kidney, intestine, and liver (Cantarow and Shepartz 1962; Horub 1982; Sherman et al. 1985; Greil et al. 1991). Some of the total unbound *myo*-inositol in brain is synthesized from glucose and the rest is transported from blood. Although the transfer rate is relatively low due to the blood-brain barrier (Cantarow and Shepartz 1962; Holub 1982; Berridge and Irvine 1989; Isaacks et al. 1994), the concentration of free *myo*-inositol in mammalian brain and cerebrospinal fluid (CSF) is higher than in plasma (Holub 1982; Greil et al. 1991). Nonetheless, in neurons the primary source of *myo*-inositol is from the recycling of PI-cycle constituents (Figure 1.2), with only about 3% of brain *myo*-inositol derived from plasma (Spector 1988).

Figure 1.2: The Phosphatidylinositol Second Messenger System (PI-Cycle). Note: The large dark circles show the location of each phosphate molecule within each respective metabolic intermediate.


CMP-PA
PA
DAG
PI
PIP
PIP$_2$
ATP
ATP

In neurons, the PI-cycle is activated following ligand binding to guanine nucleotide-binding (G_q)-protein coupled receptors, including adrenergic (α_{1A} and α_{1B}), serotonergic (5-HT_{1C} and 5-HT_2), dopaminergic (D_1), glutamatergic ($mGlu_1$ and $mGlu_5$) and cholinergic (M_1 and M_3) receptor subtypes (Fisher et al. 1992) among others (Colodny and Hoffman 1998) (Figure 1.2). More specifically, ligand binding to G_q-protein coupled receptors stimulates phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into the second messengers 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($Ins[1,4,5]P_3$ or IP_3) (Atack 2000). DAG and IP_3 each, in turn, initiate separate cascades of cellular events, including the activation of protein kinase C (PKC) and mobilization of intracellular calcium (Ca^{2+}), respectively, each of which have multiple down-stream cellular effects (Berridge and Irvine 1989; Berridge 1997; Bootman et al. 2002). Some of these down-stream cellular effects include the regulation of PKC-responsive genes (Watson and Lenox 1996), changes in neuroplasticity and gene expression (Chen and Chuang 1999), and changes in synaptic signaling (Berglund et al. 2002). The subsequent metabolism of IP_3 proceeds via two separate pathways; however, both converge on a single, common step – the dephosphorylation of IP_1 by IMPase to produce *myo*-inositol (Figure 1.2). *Myo*-inositol can also be synthesized *de novo* from G-6-P, through an IP_1 intermediate by the enzyme *myo*-inositol-1-phosphate synthase. The metabolism from IP_1 to *myo*-inositol is catalyzed by the enzyme IMPase, which is uncompetively inhibited by lithium (Li^+). *Myo*-inositol is then utilized in the resynthesis of PI. Reduced levels of myo-inositol prevent the efficient resynthesis of PI from *myo*-inositol and cytidine monophosphorylphosphatidate (CMP-PA).

With respect to brain distribution, *myo*-inositol was initially found only in astrocytes and was not observed in the neuronal cells of rat brain when measured by proton- (^1H-) and carbon- (^{13}C-) magnetic resonance spectroscopy (MRS), suggesting that *myo*-inositol may be a useful glial marker (Brand et al. 1993). In this study, less than 0.5 mM was observed in neuronal cells in comparison with ~ 6 mM of total *myo*-inositol in brain. It has been suggested that *myo*-inositol may be stored in glial cells before its consumption in the PI-cycle of neurons (Frey et al. 1998), and that astrocytes may regulate extracellular *myo*-inositol concentrations (Wolfson et al. 2000). Regionally, no differences in *myo*-inositol concentrations were reported between frontal and temporal lobes, composed of both gray and white matter (Petroff et al. 1989). Other studies have suggested that *myo*-inositol concentrations are highest in the hypothalamus and lowest in the cortex (Lubrich et al. 1997). In a post-mortem study, cerebral *myo*-inositol levels were found not to be affected by age or sex, or to change across brain regions (Shimon et al. 1997).

Given the diversity of the receptors coupled to the PI-cycle, combined with the fact that the human brain obtains most of its *myo*-inositol supply from resynthesis through the PI-cycle (Berridge et al. 1982; Sherman et al. 1985), alterations in *myo*-inositol concentration and any resultant perturbation in PI-cycle functioning (Vaden et al. 2001) may affect specific neuronal circuitry. As a result, these alterations in *myo*-inositol metabolism could have widespread effects and therefore it is conceivable that changes in PI-cycle activity may underlie many psychiatric conditions, including mood disorders. Supportive of these suggestions have been animal findings that lithium alters PI-cycle functioning, by way of a reduction in whole rat brain *myo*-inositol concentrations (O'Donnell et al. 2000). While lithium has been shown to inhibit the IMPase-driven breakdown of IP_1 to *myo*-inositol (Berridge et al. 1982), it is not clear how other medications useful in the treatment of mood disorders influence inositol levels or alter PI-cycle functioning.

1.2.1 Effects of Inositol Administration

The potential importance of PI-cycle functioning in psychiatric disorders is evident when one considers the number of receptor types/subtypes that are coupled with this signal transduction pathway. As inositol is the common precursor to all other PI-cycle constituents (Figure 1.2), maintaining a stable intracellular concentration may be important for symptom prevention and treatment in psychiatric disorders. As a result, several studies have investigated the effects of inositol administration on brain inositol levels and on changes in psychiatric symptoms in several disorders. Inositol administration has been reported to increase brain inositol concentrations in both rats (Patishi et al. 1996; Kofman et al. 1998; Pettegrew et al. 2001) and humans (Moore et al. 1999a); however, acute changes have been reported to reverse with chronic administration (Moore et al. 1999a; Pettegrew et al. 2001).

1.2.1.1 Administration in Animals

In animals, inositol administration has been shown to increase activity levels and reduce immobility time in models of depression (Einat et al. 2002, 2001, 1999a), as well as reduce anxiety-like behaviours in most (Cohen et al. 1997; Bersudsky et al. 1999; Kofman et al. 2000; Einat and Belmaker 2001; Einat et al. 1998) but not all (Cohen et al. 1996) animal models of anxiety. Similar anxiolytic effects have been reported following administration of *epi*-inositol, a non-naturally occurring stereoisomer of *myo*-inositol (Einat et al. 1998; Bersudsky et al. 1999).

1.2.1.2 Administration in Humans

In humans, inositol administration has been reported to improve depressive symptoms (assessed with the Hamilton Depression Scale) after 4 weeks compared to placebo (Levine et al. 1995; Levine 1997). Similar improvements have been reported for patients with panic disorder (Benjamin et al. 1995; Levine 1997) and obsessive-compulsive disorder (Fux et al. 1996; Levine 1997). Inositol administration was shown not to effect CSF inositol monophosphatase activity in patients with schizophrenia (Atack et al. 1998); moreover, apomorphine-induced stereotypy was not reversed following acute inositol administration (Einat and Belmaker 2001). Levine (1997) found no symptomatic improvement following inositol treatment in patients with schizophrenia and attention deficit hyperactivity disorder. Furthermore, no improvement following inositol treatment was observed in patients with post-traumatic stress disorder (Kaplan et al. 1996). In a recent study in treatment-resistent bipolar depression, antidepressant augmentation with inositol was no more effective in reducing depressive symptomatology then was augmentation with lamotrigine or risperidone (Nierenberg et al. 2006). In a study by the same group, bipolar depressed patients treated with lithium or valproate were randomly augmented with inositol or placebo (Evins et al. 2006). Patients augmented with inositol for six weeks did not differ from placebo-augmented patients in terms of their Hamilton depression scores or Young mania scores (Evins et al. 2006).

It has been noted that this spectrum of clinical utility for inositol parallels that of the SSRIs (Levine 1997; Einat and Belmaker 2001); however, the evidence that this class of drugs acts via effects on PI-cycle functioning remains weak, even though 5-HT_2 receptors are linked to the PI-cycle signal transduction pathway (Levine et al. 1999). There is no evidence to date suggesting that antidepressant medications alter inositol levels in brain. Moreover, inositol administration has been reported not to alter brain monoamine concentrations (Einat et al. 1999b), suggesting that monoamine regulation *per se* is not its mode of action. Rather, inositol may act through regulation of receptor density or modulation of downstream cellular events, including gene expression.

1.2.2 Inositol Depletion Hypothesis

At present, both the neuropathophysiology of mood disorders as well as the mechanism(s) through which current mood-stabilizer and antidepressant medications provide symptom relief are unclear. A promising avenue for research was uncovered when Allison and associates (1971, 1976) discovered that, in brain slices, lithium administration attenuates the breakdown of the inositol monophosphates into *myo*-inositol, resulting in an increase in the concentration of the inositol monophosphates and a corresponding decrease (between 20-30%) in the concentration of *myo*-inositol (Figure 1.2). As discussed above, attenuating production of *myo*-inositol slows IP_3-dependent intracellular calcium release (Wasserman et al. 2004), which in turn down-regulates the release of several neurotransmitters from synaptic vesicles, among other effects.

Building on the findings of Allison and associates (1971, 1976), Berridge and colleagues (1982) hypothesized that the clinical utility of lithium in bipolar mania may be due to *myo*-inositol depletion. Moreover, they noted that lithium's inhibition of the enzyme involved in the breakdown of IP_1 to *myo*-inositol, that enzyme being IMPase (Naccarato et al. 1974), is uncompetitive (binding to the enzyme-substrate complex), and that this inhibition occurs at therapeutically relevant plasma lithium concentrations (0.5–1.0 mEq/L) (Berridge et al. 1982). Thus, lithium would be expected to only attenuate phosphatidylinositol turnover in over-stimulated cells, through binding with the IMPase-IP_1 complex (Berridge and Irvine 1989), in effect reducing the usual neuronal responses (i.e. calcium release and related downstream effects) to receptor activation. Interestingly, lithium's actions on the PI-cycle appear to be specific to brain rather than peripheral PI-linked signaling systems, possibly due to the brain's limited access to free inositol as a result of the blood-brain barrier (Berridge and Irvine 1989) and/or because only particular neuronal populations are vulnerable to lithium (Gani et al. 1993). Any decrease in *myo*-inositol concentrations should also decrease the turnover of PIP_2 into IP_3. Such a down-regulation of agonist-stimulated IP_3 production (del Rio et al. 1998; Jenkinson et al. 1993), as well as all inositol-derived membrane phospholipids (PI, PIP, PIP_2) (Sun et al. 1992), has been reported in rat brain following lithium treatment. Moreover, Williams and colleagues (2002) reported that lithium, sodium valproate and carbamazepine all inhibit the collapse of sensory neuron growth cones and increase growth cone area, but that inositol administration reverses these effects, thus implicating inositol depletion in the action of three commonly used mood-stabilizing agents. More recent work has extended this finding to cortical neurons (Daniel et al. 2006). From these findings, it is purported that medications effective in mood-stabilization may act via inositol-depletion. The so-called inositol depletion hypothesis remains perhaps the most widely accepted hypothesis for lithium's mechanism of action in BPD (Atack 2000). By inference, this may point to hyperactivity of the PI-cycle – namely increased *myo*-inositol and inositol derivatives – in brain as a physiological factor involved in the symptom manifestation (mania and depression) of BPD. There remains a dearth of information regarding the involvment of *myo*-inositol metabolism in antidepressant action or in the symptom manifestations seen in UPD.

1.3 A Common Mechanism of Action

The validity of the inositol depletion hypothesis as an explanation of lithium's therapeutic action in BPD would be more likely if PI-cycle activity were abnormal in BPD patients. As suggested, the evidence to date supports this (reviewed in Chapter 2). However, it is much less clear that the key expectations of the inositol depletion hypothesis are correct. If lithium were to act by decreasing *myo*-inositol concentrations, it would be anticipated that these would be abnormally raised in BPD patients, and would fall following treatment. Increased *myo*-inositol concentrations were seen in the two studies of untreated manic patients (Davanzo et al. 2001, 2003). In the one study where follow-up MRS scans were carried out post-lithium, there was indeed a predicted decrease in *myo*-inositol concentrations (Davanzo et al. 2001). However, these studies were in children, and the initial finding of increased *myo*-inositol concentrations (pre-lithium treatment) was only a trend in this study, failing to meet statistical significance. The hypothesis would also predict that in manic patients treatment with lithium would also increase inositol monophosphate concentrations (contained within the phosphomonoester (PME) peak), and 3 of the four studies examining PME in manic patients treated with lithium show such an increase. Further support comes from a study in depressed patients on lithium in which *myo*-inositol concentrations were lowered (Moore et al. 2000). In three studies in depressed bipolar patients treated with lithium, the PME peak concentrations were also increased (Kato et al. 1992, 1994, 1995). However, in contrast to the manic studies, *myo*-inositol concentrations appeared lowered at baseline in a sub-analysis in the one study examining untreated depressed patients (Frey et al. 1998). Overall, therefore, with the exception of the study by Frey and associates

(1998), the data in both manic and depressed patients are supportive of the inositol depletion hypothesis.

One of the interesting findings to emerge is the possibility that both lithium and sodium valproate may have similar effects on the PI-cycle. From molecular studies, there is increasing evidence suggesting that sodium valproate may also have an effect on the PI-cycle, although this effect is not mediated via inhibition of IMPase (Vadnal and Parthasarathy 1995). In human astrocytoma cells both lithium and sodium valproate alter *myo*-inositol uptake (Wolfson et al. 2000). Both lithium and sodium valproate deplete intracellular *myo*-inositol in yeast cells (Vaden et al. 2001), while both lithium and sodium valproate can alter *myo*-inositol turnover (Li et al. 1993) and valproate can deplete inositol concentrations (Ju and Greenberg 2003). Furthermore, both lithium and sodium valproate act similarly in brain slices, leading to accumulation of IP_3, although only lithium led to the accumulation of IP_1 (Dixon and Hokin 1997). In other studies, both lithium and sodium valproate have been reported to decrease IMPase expression (Murray and Greenberg 2000), and both inhibit inositol-dependent neuronal growth cone processes (Williams et al. 2002). Additionally, lithium and sodium valproate have been shown to down-regulate PKC activity in rat brain (Manji and Lenox 1994) and more recently in the platelets of manic BPD patients (Hahn et al. 2005). While molecular evidence exists, to date, the majority of the MRS studies have focused primarily on lithium treatment, with only a few studies assessing the effects of sodium valproate on PI-cycle functioning *in vivo*. In euthymic BPD patients, two studies reported that treatment with either lithium or sodium valproate did not alter *myo*-inositol or PME levels (Silverstone et al. 2002; Chang et al. 2003). Sodium valproate has been shown to have no effect on *myo*-inositol levels in a group of depressed (Moore et al. 2000) as well as manic and mixed (Cecil et al. 2002) BPD patients. Sodium valproate has also been shown to reduce *myo*-inositol and increase IP_1 concentrations in whole rat brain, similar to those changes that occurred following lithium administration (O'Donnell et al. 2000). In terms of determining if alteration of PI-cycle activity could be a common mechanism of action, to date there are no MRS studies examining the effects of treatment with other commonly prescribed mood-stabilizing agents such as lamotrigine and carbamazepine. Nor is there sufficient research assessing the clinical and preclinical effects of antidepressants on PI-cycle activity.

1.4 Nuclear Magnetic Resonance

Twenty-five years ago, Ackerman and associates (1980) published the first nuclear magnetic resonance (NMR) spectrum (phosphorus-31 or ^{31}P) from a living brain (rat), ushering in a new era in the study of brain metabolism. The first *in vivo* ^{1}H spectrum from brain was published a few years later (Behar et al. 1983). Over the ensuing two decades, numerous preclinical (*in vivo* and *in vitro*), as well as clinical (*in vivo*) applications of MRS for the study of brain neurochemistry and amino acid metabolism have been reported (Williams 1999). Today, several neuroimaging modalities, including conventional magnetic resonance imaging (MRI), MRS, functional MRI (fMRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT) all provide a means to help better elucidate the structural (MRI), neurochemical (MRS) and functional (fMRI, PET and SPECT) correlates of human and animal neurophysiology. The characteristics and attributes of these neuroimaging modalities are summarized and contrasted in Table 1.1.

Table 1.1: Brain Imaging Technologies in Mood Disorders. [Reprinted with permission from: Anand and Shekhar (2003)].

	MRI	MRS	fMRI	PET/SPECT
Radiation exposure	No	No	No	Yes
Availability	Widely available	Selected centres	Widely available	Selected centres
Cost	Low	High	High	High
Resolution	High	Low	Low	High with PET; Low with SPECT
Imaging in pediatric population	Yes	Yes	Yes	No
Motion artifacts	Low	High	High	Medium
Absolute quantification	High	Medium	Medium	High
Signal/noise ratio	High	Low	Low	Medium
Brain regions that can be studied	All	Limited number of regions per session	Regions near sinuses difficult to quantify	Depending on radiotracer binding, more success with radiotracers that bind to specific regions, e.g., stratium
Strength of biological significance of measures	High	Low	Medium	Medium
Resting state measurement	Yes	Yes	No	Yes
Functional (event-related activation) imaging	No	No	Yes	Yes
Hemodynamic (blood flow) imaging	No	No	Yes	Yes
Neuronal metabolism measurement	No	Yes	Yes (indirect)	Yes (indirect)
Neurochemical imaging	No	Yes, limited to metabolites of high concentration	No	Yes, limited to radiotracer availability
Neuroreceptor imaging	No	No	No	Yes

Although other imaging modalities exist for the study of brain neurochemistry and metabolism, MRS is the only available technique that is non-invasive and that can be utilized both in the clinical (*in vivo*) as well as the preclinical (*in vivo* and *in vitro*) milieu. Compared with *in vivo* MRS, *in vitro* (or high field) MRS has a higher spectral resolution and sensitivity, due primarily to the stronger static magnetic field and the use of smaller, more homogeneous samples (Burri et al. 1990).

The physical-chemical properties of brain tissue facilitate the use of NMR spectroscopic techniques for the investigation of neurochemistry. More specifically, these properties include the presence of compounds or molecules (i.e. water, amino acids, proteins and lipids, among others) that contain magnetically active nuclei (Desmond and Tress 1997), with ^{1}H and ^{31}P being two of the most common nuclei species investigated in the neurosciences, contributable primarily to their isotopic abundance. When placed in the static magnetic field (B_0), the nuclei align parallel or anti-parallel to the applied field. The application of a second, brief and alternating magnetic field (in the form of a radiofrequency pulse) causes the nuclei to become excited. Excitement occurs only if the applied radiofrequency pulse matches the Larmor (or precessional) frequency (ω_0) for a particular nuclei at its gyro-magnetic ratio (γ) (or the ratio of the magnetic dipole moment to the angular momentum of an elementary particle or atomic nucleus) at a given static field strength (B_0). This relationship is described by the Larmor equation, where $\omega_0 = \gamma B_0$ (Cowan 1997). In this environment, the excited nuclei absorb the additional energy and reverse their spin direction. Alternating the application of this second magnetic field causes the nuclei to relax back to their resting state, emitting the absorbed energy, which is detectable in the radiofrequency range (Farrar 1990). The heterogeneous nature of the neurochemical environment means that each nucleus will interact with various internal and external magnetic fields in addition to the surrounding electronic magnetic fields (Allen 1990). Under these influences, each signal observed is thus characteristic of the frequency at which a given nucleus resonates back to its resting state, commonly referred to as the chemical shift.

Utilizing the same basic principles as MRI, MRS goes beyond just assessment of structure, providing biochemical and metabolic data from brain regions (or volumes) of interest in the study of neurological diseases, psychiatric disorders (Brandao and Dominques 2004) and central drug action. Moreover, quantitation of MRS-visible metabolites can help investigators understand both the role(s) of specific neurochemicals in normal and pathological states, as well as in the action of neurotropic agents on specific neurochemicals (Govindaraju et al. 2000). When placed in a strong magnetic field and penetrated by an electromagnetic wave in the radiofrequency (rf) range (for proton: 127.50 megahertz (MHz) @ 1.5 tesla (T) to 800MHz @ 18.8T), relative quantification of these metabolites can be determined (in relation to a reference compound) and depicted as chemical shift spectra (in parts per million, ppm), with the area under the curve representing the relative concentrations of the metabolites of interest. From a clinical perspective, this means that with currently available magnetic field strengths (1.5T to 7T) several (~6) biogenic compounds and amino acid neurotransmitters (including *myo*-inositol, *N*-acetylasparate, choline, creatine+phosphocreatine, GABA and glutamate) are MRS detectable. In the preclinical milieu, the use of even higher field strengths (9T to 18.8T) enables detection and identification of numerous (~25) metabolites from small amounts of brain extract.

The next several sections characterize the application of both clinical and ultra high-field MRS in the one-dimensional study of *in vivo* and *in vitro* neurochemistry, respectively. As the majority of the research to date has utilized the proton (^{1}H) nucleus – due primary to its high sensitivity and natural abundance – and given that the present body of work utilizes this nucleus, ^{1}H-MRS will be the focus of the present discussion. More specifically, these sections will discuss the limitations of clinical MRS and the novel application of a NMR sequence specifically designed for the detection of *myo*-inositol *in vivo* at 3T.

The novel application of 18.8T proton NMR spectroscopy to the study of *in vitro* neurochemistry in rats, including the utilization of a recently developed NMR spectroscopy profiling software and the technical aspects of sample preparation as well as acquiring and characterizing spectra obtained from brain tissue at high-field strengths *in vitro* will also be discussed.

1.4.1 In vivo Proton (^1H) NMR Spectroscopy

The newness of the technology combined with the debilitating course of any psychiatric illness, urgency of treatment and the stringent requirements of any well-designed investigation have lead to two common, but significant, limitations in the MRS studies done to date. These limitations are the measurement of metabolite change as a function of a ratio between two metabolites and the use of low field strength magnets.

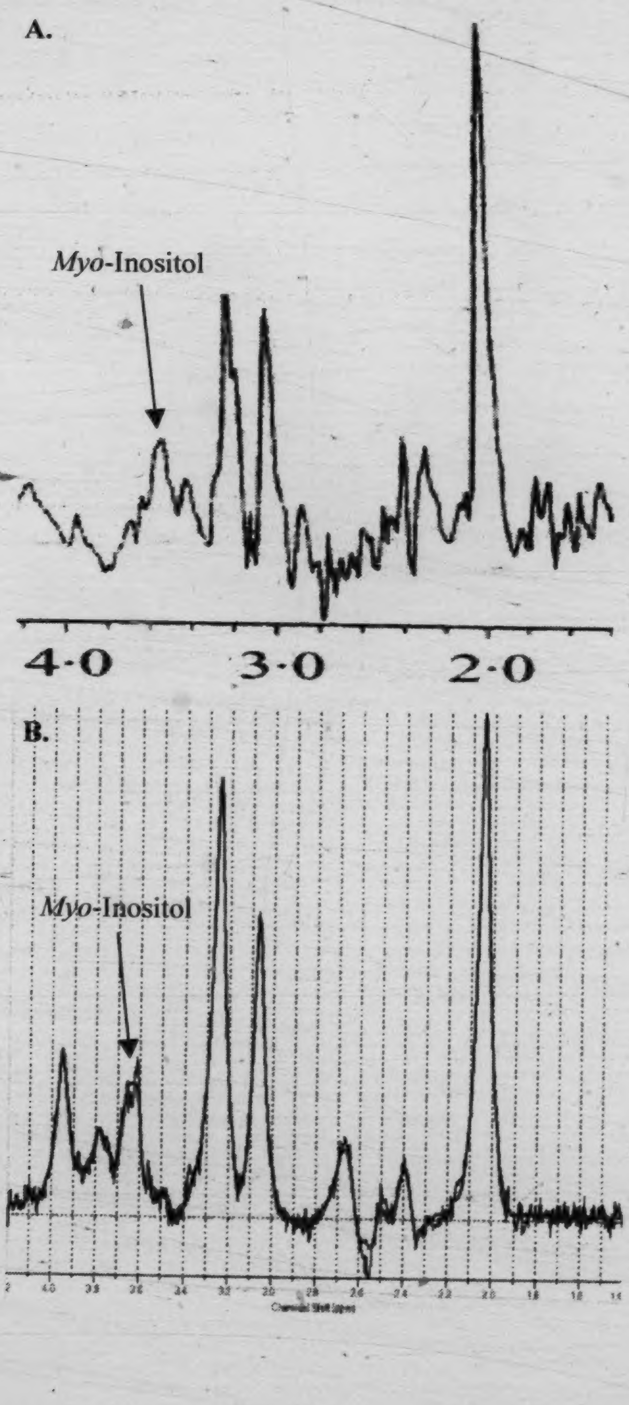
1.4.1.1 Limitations in quantification

From a purely technical standpoint, two areas in the current clinical MRS literature warrant scrutiny and improvement. First, the vast majority of the studies have relied on the measurement of metabolite change as a ratio between the metabolite of interest (numerator) and some internal standard (denominator). In ^{1}H MRS, the most commonly used internal standard is the creatine+phosphocreatine peak (Cr-PCr). *N*-acetylaspartate (NAA) has also been used in many studies, as it is the most prominent (or largest) peak at clinical field strengths. Irrespective of the metabolite used, the same criticism applies. That is, this approach to quantification relies on the assumption that the standard comparator peak is unchanging, an assumption whose validity has recently been called into question. As a case in point, Hamakawa and associates (1999) found that Cr concentrations are significantly reduced in the frontal lobe of BPD depressed, when compared to euthymic, patients. Similiarly, Kato and colleagues (1995) have reported significantly lower concentrations of PCr in the frontal lobe of lithium-treated BPD-II patients, negatively correlating this decrease with scores on the Hamilton Rating Scale for Depression. These changes in PCr levels were not found in BPD-I patients (Davanzo et al. 2003), possibly indicating a difference in energy metabolism between the two BPD subtypes. Finally, Deicken and colleagues (2001) have reported higher Cr concentrations in the anterior and mediodorsal thalamic regions in euthymic BPD patients. Moreover, other factors related to illness, treatment, age and gender might affect the reliability of this approach. As a result, the development of an approach that uses brain water concentration, or an external standard of known concentration, as the reference peak is warranted. The present body of work improves upon this limitation and reports results as ratios to brain water.

1.4.1.2 Field Strength

The second technical limitation has been the use of low field strength magnets, with most studies in the mood disorders literature utilizing 1.5T and 2T magnets. Of concern is the low chemical shift dispersive power, often resulting in peak overlap between individual metabolites of interest, as in the case of *myo*-inositol and glycine, and the glutamate+glutamine (Glx) peak, which contains contributions from glutamate, glutamine and GABA. Newly available magnets with higher field strengths, including 3T, 4.7T and even 8T, as well as the optimization of pulse sequences specifically targeting individual metabolites of interest can provide better spatiotemporal resolution and higher sensitivity, which is key to any future advances in clinical spectroscopic investigation (Figure 1.3).

Figure 1.3: Spectra Acquired at 1.5T (A: Hamakawa et al. 1999) and 3T (B: from current study) from the Frontal Cortex. Region from 1.4 – 4.2 ppm shown, with the peak corresponding to *myo*-inositol indicated at about 3.56 ppm.



The present body of work improves upon this limitation by utilizing a 3T magnet and by employing a novel and newly developed pulse sequence specially designed for the optimized detection of *myo*-inositol (Kim et al. 2005). This approach is expected to increase the stability and accuracy of the spectroscopy results, providing more precise measurement of brain *myo*-inositol concentrations.

1.4.2 High-Field In vitro Proton (^1H) NMR Spectroscopy

High Field *in vitro* NMR spectroscopy has been utilized for decades in organic chemistry as a technique for the identification of synthetic products (Hall 1964). In the late 1980s it was discovered that NMR could be also used for the determination of three-dimensional structures of macro biomolecules (e.g. small proteins, enzymes, DNA, RNA, and bio-complexes) in solution and the elucidation of protein structure/function relationships (e.g. pH changes in enzymatic active sites) (Berliner and Wan 1989; Ishida et al. 1987; Dalvit and Wright 1987; Dohn et al. 1985). Today NMR is used on samples in both liquid and solid state, and on a wide variety of nuclei ranging in application disciplines from biochemistry, molecular biology, neuroscience, inorganic and organic chemistry, nanotechnology, industrial compounds, to even quantum computing.

The primary advantage afforded by *in vitro* MRS analysis of brain tissue is the availability of magnets with field strengths several orders of magnitude greater then those available for *in vivo* investigation (i.e. 3T versus 18.8T) (Arus et al. 1985). As a general rule, as the magnetic field strength increases, so too does the resolution between metabolite resonant frequencies and the signal-to-noise ratio (Salibi and Brown 1998), enabling the accurate identification of a greater number of MRS visible compounds. In this way, *in vitro* MRS at high field strengths eliminates the problems more often encountered in the clinical milieu. Moreover, *in vitro* MRS provides a higher throughput, contributable primarily to the greater homogeneity of the sample and magnetic field. That said, utilizing *in vitro* MRS requires sample preparation prior to analysis, as detailed below.

In vivo MRS requires little by way of actual sample preparation. And although *in vivo* assessment offers the opportunity to study an intact neurochemical environment, it is not without drawbacks. In clinical investigations, subject movement is a common concern and can lead to movement artifacts and unusable data. In preclinical studies of intact animals movement is less of a concern, as the animals are often anesthetized and restrained. However, anesthesia introduces another problem, in that it may alter the neurochemical environment in unknown ways. Finally, both clinical and preclinical *in vivo* approaches require more time for data collection, have a lower throughput, a higher cost per unit time, utilize magnets with lower field strengths and do not enable the absolute quantification of brain metabolites. An improvement in all these areas can be achieved with the use of *in vitro* MRS.

1.4.2.1 Magnetic Field Strength

The NMR signal intensity, resolution, and utility are directly proportional to the magnitude, homogeneity, and consistency, respectively, of the external magnetic field applied during the experiment (Macomber 1998). Most high field spectrometers are super conducting static magnets providing stable fields in excess of 100,000 to 180,000 times the magnetic field of the earth. Similar to magnets used in the clinical milieu, these fields are completely benign for samples as well as spectrometer operators providing a unique, non-invasive method for quantitative and qualitative studies at the atomic level.

1.4.2.2 Spectroscopy at 800 MHz

The 18.8 T (800 MHz ^{1}H resonance frequency) spectrometers are the largest and most stable of the ultra high-field magnets commonly available today. Larger magnets exist (i.e. 900 MHz and even 1GHz) but these are not commonly available (~ a dozen world wide) nor do they contain the inherent field stability/performance characteristics of the 800 MHz spectrometers (R McKay, personal communication). Spectrometers are considered established or mainstream when they are available with a non-pumped 4.2 Kelvin (K) cryostat (magnet coil charge density does not exceed liquid helium cooling capacities) and active field shielding for a reduced laboratory footprint. This criterion has recently been made available for magnets at the 800 MHz frequency. To date, there have been no published reports of metabolite data from brain extract (rat or otherwise) acquired at 18.8T.

The 18.8T magnet is also ideal for low concentration metabolite studies due to the recent development of cryogenically cooled radiofrequency probe electronics (commonly referred to as cryoprobes or cold probes) for this magnetic field strength spectrometer. The cold-probe can yield a signal-to-noise (S/N) ratio increase by a factor of 2-4 over conventional room temperature NMR probes (Bradley et al. 2005). The performance increase is inversely proportional to the salt concentration of the sample in question and also depends greatly on the inherent quality of the magnet and the magnet's environment. As with other Fourier transform techniques, repeated sample yields an increase in the NMR S/N ratio. The square root of the number of collected transients determines the relative increase (e.g. increasing the number of cumulative experiments by a factor of 4 results in a S/N increase of a factor of 2) in the S/N achieved (Macomber 1998). Thus an increase of 2-4 in the S/N by the use of a cold-probe can either yield identical data in vastly reduced time periods (4-16 fold less time), or can achieve usable spectra of otherwise impractically low concentration samples. In the present body of work, this provides an advantage given the number of samples (several hundred) that must be run and the relatively low metabolite concentrations (micromole range) of each sample. With the cold-probe, usable data can be collected on metabolite concentrations as low as 100-1 micromole or even into the nanomolar range; however, this does require extended acquisition periods (hours instead of seconds). Spectrometers of lower field strength make it impractical to assess small brain nuclei (e.g. amydala and nucleus accumbens) requiring instead the evaluation of whole brain concentrations.

1.4.2.3 Field and Sample Shimming

Slight imperfections in the uniformity of the spectrometer magnetic field can be compensated for with small user manipulated magnetic fields inside the magnet core referred to as "shims". The act of altering these compensatory fields is called "shimming" and requires a certain degree of uniformity in the sample (e.g. particulate matter is too large to be compensated for while visually imperceptible imperfections in the NMR tube glass tube can be) and operator skill. The most obvious result of poor field homogeneity is adverse solvent suppression in the NMR sample. Most samples are dissolved in H_2O that results in an enormous signal (55 molar (M)) when compared to millimolar (mM) (or lower) solute resonances. This is indeed the case for brain extract. In these cases NMR techniques can be used to suppress the solvent signal while retaining the remaining information of interest. The efficacy of suppressing the solvent resonances is directly proportional to the quality of the magnet field (i.e. the uniformity and resolution, or width of the solvent resonance). Alternatively, samples can be dissolved in deuterium oxide (D_2O), which is not NMR visable but does require accounting for the small differences in chemical shift due to the solvent isotope effect (Govindaraju et al. 2000). Furthermore, inclusion of some amount of D_2O is often necessary to monitor and maintain the stability of the spectrometer, which is done by monitoring the deuterium signal. A solvent combination of water (H_2O) and D_2O is also possible, and was the approach employed in the present set of experiments.

1.4.2.4 Acquisition Parameters

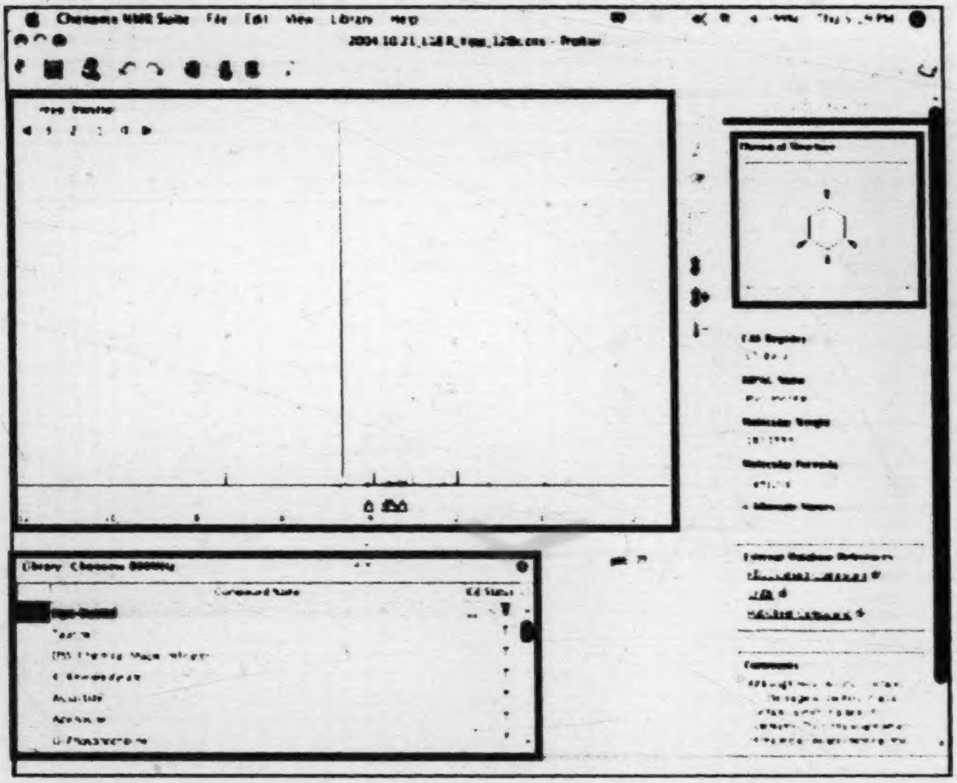
As mentioned above, a higher S/N ratio can be achieved by repeating the sampling, and summing the resulting signals. This is based on the premise that real signal will add, while random error will average out (Sanders and Hunter 1997). Equilibrium of the sample must be re-established before the next excitation energy NMR pulse can be efficiently imparted. As the time required to establish equilibrium is an exponential (i.e. more and more time is required to reach further equilibrium), a relaxation delay is typically selected by the spectrometer operator to provide maximum recovery in the least amount of time (i.e. 3-5 times the relaxation rate constant, which was around 1.5 seconds for samples of brain extract).

The relaxation delay used in these experiments was extended beyond that required for equilibrium and used to more effectively pre-saturate the water signals. This allowed selective removal of a majority of the solvent resonance from the free induction decays (R McKay, personal communication).

1.4.2.5 Spectral Analysis and Metabolite Quantification

Once collected, spectra need to be interpreted and identified metabolites need to be quantitated. In the present set of preclinical experiments, Chenomx NMRSuite v.4.0 was used for metabolite identification and quantitation in the rat brain extracts (Figure 1.4). Chenomx NMRSuite Profiler has a compound library of over 200 unique spectral signatures that can be overlayed onto the rat brain NMR spectra to deconvolute based upon the compound peak patterns. By adding a known amount of the internal standard, such as 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), the concentrations of the metabolites may be determined relative to this standard. Absolute brain concentrations based on wet tissue weight can then be determined.

Figure 1.4: Screenshot from Chenomx NMRSuite Profiler v.4.0.



The blue box displays the individual spectrum as well as the overlay of the metabolite being quantitated (*myo*-inositol in this instance).

The red box displays the structure of the metabolite that is being quantitated.

The box displays chemical information about the metabolite that is being quantitated.

The green box displays the list of all metabolites contained in the software library, as well as the concentration of the metabolites once quantitated.

1.4.2.6 Metabolite Identification

Table 1.2 shows the list of metabolites identified and quantified in the rat brain spectra using the Chenomx NMRSuite software. The Chenomx compound libraries have encoded the information relating to the chemical shifts, multiplicities, and relative intensities of the peaks and peak clusters for compounds in solution at a pH of approximately 7. Identification proceeds in a straightforward manner, by matching the unique spectral patterns of each metabolite to the actual spectrum. The chemical shifts of each peak cluster may be manually adjusted within a defined window to optimize the fitting process. At 18.8T *myo*-inositol produces four multiplets centered at 3.27, 3.52, 3.62 and 4.05 ppm (Table 1.2 and Figure 1.5). The most prominent signals come from the doublet of doublets centered at 3.52 and the triplet centered at 3.62 ppm (Table 1.2 and Figure 1.5).

Table 1.2: ¹H Chemical Shifts of Neurochemicals Detected in Rat Brain Extract at 18.8T.

	Compound	Proton Type	Multiplicity	Number of Protons (Relative Intensity)	¹H Chemical Shift (ppm)
1	Glutamate	αCH	dd	1	3.75
		γCH_2	m	2	2.35
		βCH-1	m	1	2.12
		βCH-2	m	1	2.04
2	Lactate	CH	q	1	4.10
		CH_3	d	3	1.31
3	Creatine	CH_2	s	2	3.91
		CH_3	s	3	3.03
4	N-Acetylasparate	NH	d	1	7.82
		αCH	m	1	4.38
		βCH-1	dd	1	2.68
		βCH-2	dd	1	2.49
		CH_3	s	3	2.01
5	Glutamine	NH_2	s	2	6.81
		αCH	t	1	3.76
		γCH_2	m	2	2.45
		βCH_2	m	2	2.12
6	Aspartate	CH	dd	1	3.89
		CH-1	dd	1	2.81
		CH-2	dd	1	2.65
7	Taurine	SCH_2	t	2	3.42
		NCH_2	t	2	3.25

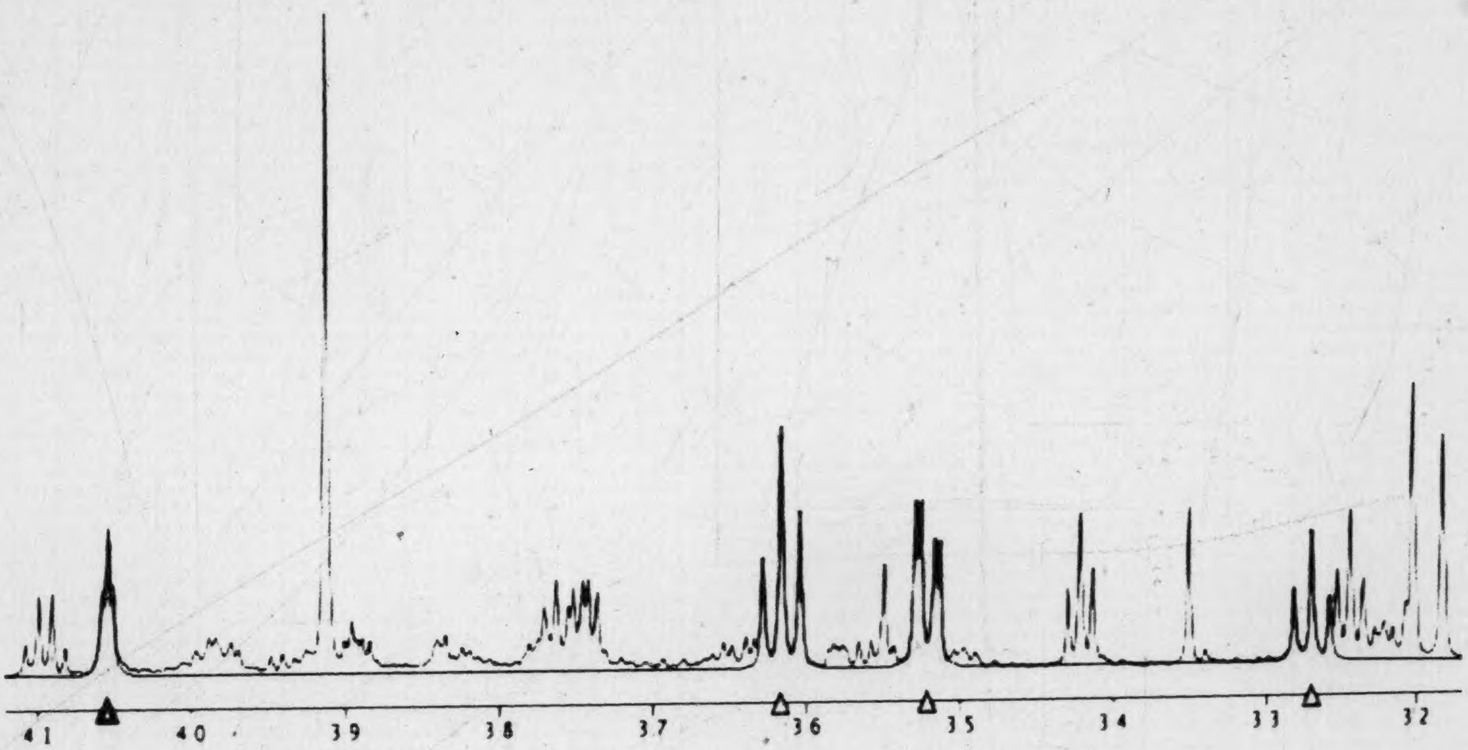
	Compound	Proton Type	Multiplicity	Number of Protons (Relative Intensity)	1H Chemical Shift (ppm)
8	Myo-Inositol	CH1	t	1	4.05
		CH2,6	t	2	3.62
		CH3,5	dd	2	3.52
		CH4	t	1	3.27
9	γ-Aminobutyric Acid	αCH_2	t	2	3.01
		γCH_2	t	2	2.29
		βCH_2	t	2	1.89
10	Hypoxanthine	CH-2	s	1	8.20
		CH-8	s	1	8.18
11	Adenosine	CH-8	s	1	8.32
		CH-2	s	1	8.22
		CH-1'	d	1	6.08
		CH-2'	dd	1	4.78
		CH-4'	dd	1	4.42
		CH-3'	dd	1	4.26
		CH-5'	dd	1	3.90
		CH-5"	dd	1	3.83
12	Serine	βCH-1	dd	1	3.98
		βCH-2	dd	1	3.94
		αCH	dd	1	3.84
13	Glycine	αCH_2	s	2	3.55
14	Alanine	αCH	q	1	3.78
		βCH_3	d	3	1.47

	Compound	**Proton Type**	**Multiplicity**	**Number of Protons (Relative Intensity)**	**1H Chemical Shift (ppm)**
15	Phosphorylcholine	OCH_2	m	2	4.17
		NCH_2	m	2	3.58
		$3CH_3$	s	9	3.20
16	Choline	OCH_2	m	2	4.05
		NCH_2	m	2	3.50
		$3CH_3$	s	9	3.18
17	Threonine	βCH	m	1	4.25
		αCH	d	1	3.58
		γCH_3	d	3	1.32
18	Acetate	CH_3	s	3	1.91
19	Leucine	αCH	dd	1	3.73
		βCH_2, γCH	m	3	1.70
		δCH_3-1	d	3	0.95
		δCH_3-2	d	3	0.94
20	Niacinamide	CH-2	s	1	8.93
		CH-4	d	1	8.70
		CH-6	d	1	8.24
		CH-5	dd	1	7.59
21	Formate	CH	s	1	8.44
22	Valine	αCH	d	1	3.60
		βCH	m	1	2.26
		γCH_3-1	d	3	1.03
		γCH_3-2	d	3	0.98
23	Succinate	2-CH_2	s	4	2.40

	Compound	**Proton Type**	**Multiplicity**	**Number of Protons (Relative Intensity)**	**1H Chemical Shift (ppm)**
24	Fumarate	2CH	s	2	6.50
25	Uracil	CH5	d	1	7.52
		CH6	d	1	5.79
26	Histidine	CH2	s	1	7.86
		CH4	s	1	7.09
		αCH	dd	1	3.98
		βCH-1	dd	1	3.23
		βCH-2	dd	1	3.13
27	Isoleucine	αCH	d	1	3.66
		βCH	m	1	1.97
		γCH$_2$-1	m	1	1.45
		γCH$_2$-2	m	1	1.25
		γCH$_3$	d	3	1.00
		δCH$_3$	t	3	0.93

Abreviations: s=singlet, d=doublet, dd=doublet of doublets, m=multiplet, q=quartet.

Figure 1.5: Typical Spectrum acquired from rat brain extract (black). Region displayed is from 3.2 – 4.1 ppm with the Chenomx Profiler peak fit for *myo*-inositol shown in blue.



1.4.2.7 Metabolite Quantification

Upon addition of a known concentration of an internal standard, in this case DSS, all identified metabolites present in the brain extracts at micromolar concentration and above may be simultaneously quantified using NMR spectroscopy. NMR is inherently quantitative in nature as the areas under each peak cluster directly correspond to the compound concentration in a predictable way assuming the chemical structures are known (Govindaraju et al. 2000). For example, the concentration of a compound may be determined by comparing the area of one of its peak clusters to the area of a known peak cluster. Theoretically, for ^{1}H NMR, the determination of the concentration of the unknown compound simply involves the multiplication of the area under the peak cluster by the number of protons resonating at that frequency divided by the area of the reference compound that has been multiplied by the number of protons resonating at its frequency (Gunther 1995). However, small molecule NMR is complicated by long relaxation times, which means that the above statement will not yield precise quantitation. For this reason, in the Profiler software, libraries of metabolites have been created under certain NMR experimental conditions that if followed will give accurate analysis of all metabolite concentrations in the micromolar (μM) range and higher (C Slupsky, personal communication). To ensure that these reference spectra correspond with the spectra obtained from the sample, it is important that the NMR acquisition parameters and experimental conditions (i.e. sample pH and temperature) be as similar as possible (Govindaraju et al. 2000). Adding the internal standard and having the standard present in the mixture negates the prospect of needing to calibrate the instrument. Furthermore, NMR spectroscopy has been shown to yield concentration values identical or very similar to those reported in the chromatographic literature (Burri et al. 1990, O'Donnell et al. 2003). A major advantage of NMR is that unknown compounds may still be identified and quantified without the need to re-run the sample, risking degradation as the sample is stored over time, as the NMR data are stored on computer and may be analyzed and re-analyzed as more metabolites are identified and added to the compound library.

Major sources of error in quantitation, assuming the spectra have been collected under similar parameters, include modulation in the peak shapes and spectral baseline that result from poor shimming during data acquisition, and the presence of protein and lipid contaminants. The extract method employed in the present set of experiments was designed to separate out these protein and lipid contaminants (Bligh and Dyer 1959). In addition, to obtain optimal spectral quality, the spectra were also zero-filled to 128k datapoints while also employing a linebroadening apodization function of 0.5 Hz during the spectral processing in an effort to smooth the data.

In summary, proton MRS is particularly well suited to provide useful biochemical information from brain, including information on brain development, disease states and drug effects. The size of the brain region, the natural abundance of a given metabolite and the strength of the magnet all influence the ability to detect a given metabolite in a given brain region (Govindaraju et al. 2000; Pfeuffer et al. 1999). Clinical neuroimaging, particularly using NMR-based approaches, is one of the most important advances for the *in vivo* study of brain structure, function and chemistry. This methodology has provided, for the first time, a non-invasive (in the case of NMR) approach for examining human neurophysiology. Similarly, preclinical MRS at high field strengths provides a similar means of studying modeled conditions or the effects of treatment at greater resolution. This is also one of only a handful of methodological tools that can be applied to both the *in vivo* and *in vitro* study of human and animal neurophysiology.

1.5 Bibliography

Ackerman JJ, Grove TH, Wong GG, Gadian DG, Radda GK. Mapping of metabolites in whole animal by 31P NMR using surface coils. Nature 1980;283:167-170.

Adams F. The Extant Works of Aretaeus, the Cappadocian. Sydenham Society: London, UK. 1856.

Ainsworth K, Smith SE, Sharp T. Repeated administration of fluoxetine, despiramine and tranylcypromine increases dopamine D2-like but not D1-like receptor function in the rat. J Psychopharmacol 1998;12:252-257.

Akiskal HS. Classification, diagnosis and boundaries of bipolar disorders: A review. In: WPA Series, Evidence and Experience in Psychiary, Volume 5: Bipolar Disorder. Maj M, Akiskal HS, Lopez-Ibor JJ, Sartorius N (eds.). John Wiley & Sons: London, UK. 2002.

Akiskal HS, Bourgeois ML, Angst J, Post R, Moller H, Hirschfeld R. Re-evaluating the prevalence of and diagnostic composition within the broad clinical spectrum of bipolar disorders. J Affect Disord 2000;59(Suppl 1):S5-S30.

Akiskal HS, Pinto O. The evolving bipolar spectrum. Prototypes I, II, III, and IV. Psychiatr Clin North Am 1999;22:517-534.

Alda M. The phenotypic spectra of bipolar disorder. Eur Neuropsychopharmacol 2004;14:S94-S99.

Allen PS. In vivo magnetic resonance spectroscopy applied to medicine. J Can Assoc Radiol 1990;41:39-44.

Allison JH, Blisner ME, Holland WH, Hipps PP, Sherman WR. Increased brain myo-inositol 1-phosphate in lithium-treated rats. Biochem Biophys Res Comm 1976;71:664-670.

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nat New Biol 1971;233:267-268.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revised. American Psychiatric Publishing Group: New York, NY. 2000.

Amsterdam JD, Brunswick DJ. Antidepressant monotherapy for bipolar type II major depression. Bipolar Disord 2003;5:388-395.

Anand A, Shekhar A. Brain imaging studies in mood and anxiety disorders: Special emphasis on the amygdala. Ann N Y Acad Sci 2003;985:370-388.

Angst J. The emerging epidemiology of hypomania and bipolar II disorder. J Affect Disord 1998;50:143-151.

Angst J. The epidemiology of depressive disorders. Eur Neuropsychopharmacol 1995;5 Suppl:95-98.

Arus C, Yen-Chang, Barnay M. Proton nuclear magnetic resonance spectra of excised rat brain. Physiol Chem Phys Med NMR 1985;17:23-33.

Aston-Jones G, Rajkowski J, Cohen J. Role of locus coeruleus in attention and behavioural flexibility. Biol Psychiatry 1999;46:1309-1320.

Atack JR. Lithium, phosphatidylinositol signaling, and bipolar disorder: The role of inositol monophosphatase. In: Bipolar Medications: Mechanisms of Action. Manji HK, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Atack JR, Levine J, Belmaker RH. Cerebrospinal fluid inositol monophosphatase: elevated activity in depression and neuroleptic-treated schizophrenia. Biol Psychiatry 1998;44:433-437.

Ayuso-Gutierrez JL, Ramos-Brieva JA. The course of manic-depressive illness. A comparative study of bipolar I and bipolar II patients. J Affect Disord 1982;4:9-14.

Baldassano CF, Datto SM, Littman L, Lipari MA. What drugs are best for bipolar depression? Ann Clin Psychiatry 2003;15:225-232.

Bearden CE, Hoffman KM, Cannon TD. The neuropsychology and neuroanatomy of bipolar affective disorder: A critical review. Bipolar Disorders 2001;3:106-150.

Behar KL, den Hollander JA, Stromski ME, Ogino T, Shulman RG, Petroff OA, Prichard JW. High-resolution 1H nuclear magnetic resonance study of cerebral hypoxia in vivo. Proc Natl Acad Sci USA 1983;80:4945-4948.

Benazzi F. Bipolar II disorder family history using the family history screen: Findings and clinical implications. Compreh Psychiatry 2004;45:77-82.

Benjamin J, Levine J, Fux M, Aviv A, Levy D, Belmaker RH. Double-blind, placebo-controlled, crossover trial of inositol treatment for panic disorder. Am J Psychiatry 1995;152:1084-1086.

Berglund K, Midorikawa M, Tachibana M. Increase in the pool size of releasable synaptic vesicles by the activation of protein kinase C in goldfish retinal bipolar cells. J Neurosci 2002;22:4776-4785.

Berliner LJ, Wan XM. In vivo pharmacokinetics by electron magnetic resonance spectroscopy. Magn Reson Med 1989;9:430-434.

Berns GS, Nemeroff CB. The neurobiology of bipolar disorder. Am J Med Genet 2003;123C:76-84.

Berridge MJ. The 1996 Massry Prize. Inositol trisphosphate and calcium: two interacting second messengers. Am J Nephrol 1997;17:1-11.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Berridge MJ, Downes CP, Hanley MR. Neural and developmental actions of lithium: a unifying hypothesis. Cell 1989;59:411-419.

Berridge MJ, Irvine RF. Inositol phosphates and cell signaling. Nature 1989;341:197-205.

Bersudsky Y, Einat H, Stahl Z, Belmaker RH. Epi-inositol and inositol depletion: two new treatment approaches in affective disorder. Curr Psychiatry Rep 1999;1:141-147.

Bezchlibnyk Y, Young LT. The neurobiology of bipolar disorder: Focus on signal transduction pathways and the regulation of gene expression. Can J Psychiatry 2002;47:135-148.

Birchall J and Chappell J. Aluminum, chemical physiology, and Alzheimer's disease. Lancet 1988;29:1008-1010.

Blier P. Possible neurobiological mechanisms underlying faster onset of antidepressant action. J Clin Psychiatry 2001;62(Suppl 4):7-11.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-917.

Bootman MD, Berridge MJ, Roderick HL. Calcium signalling: more messengers, more channels, more complexity. Curr Biol 2002;12:R563-565.

Bowden C, Brugger A, Swann A, Calabrese JR, Janicak PG, Petty F, Dilsaver SC, Davis JM, Rush AJ, Small JG. Efficacy of divalproex vs lithium and placebo in the treatment of mania. The Depakote Mania Study Group. JAMA 1994;271:918-924.

Bradley SA, Paschal J, Kulanthaivel P. DOSY of sample-limited mixtures: Comparison of cold, nano and conventional probes. Magn Reson Chem 2005;43:31-35.

Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. Dev Neurosci 1993;15:289-298.

Brandao LA, Dominques RC. MR Spectroscopy of the Brain. Lippincott Williams & Wilkins: New York, NY. 2004.

Burri R, Bigler P, Straehl P, Posse S, Colombo J-P, Herschkowitz N. Brain development: ^{1}H magnetic resonance spectroscopy of rat brain extracts compared with chromatographic methods. Neurochem Res 1990;15:1009-1016.

Byrne SE, Rothschild AJ. Loss of antidepressant efficacy during maintance therapy: Possible mechanisms and treatments. J Clin Psychiatry 1998;59:279-288.

Cade J. Lithium salts in the treatment of psychotic excitement. Med J Australia 1949;36:349-352.

Calabrese JR. Efficacy of lamotrigine in bipolar disorder. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Calabrese JR, Bowden CL, Sachs G, Yatham LN, Behnke K, Mehtonen OP, Montgomery P, Ascher J, Paska W, Earl N, DeVeaugh-Geiss J, Lamictal 605 Study Group. A placebo-controlled 18-month trail of lamotrigine and lithium maintenance treatment in recently depressed patients with bipolar I disorder. J Clin Psychiatry 2003;64:1013-1024.

Calabrese JR, Bowden CL, Sachs G, Ascher JA, Monaghan E, Rudd GD, Lamictal 602 Study Group. A double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. J Clin Psychiatry 1999;60:79-88.

Cantarow A, Schepartz B. Biochemistry. WB Saunders Company: London, UK. 1962.

Cecil KM, DelBello MP, Morey R, Strakowski SM. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord 2002;4:357-365.

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children and familial bipolar disorder. Biol Psychiatry 2003;53:1059-1065.

Chen G, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. J Biol Chem 1999;274:6039-6042.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001;50:260-265.

Cleare AJ, Murray RM, O'Keane V. Do noradrenergic reuptake inhibitors affect serotonergic function in depression? Psychopharmacol 1997;134:406-410.

Clerc GE, Ruimy P, Verdeau-Pailles J, on behalf of the Venlafaxine French Inpatient Study Group. A double-blind comparison of venlafaxine and fluoxetine in patients hospitalized for major depression and melancholia. Int Clin Psychopharmacol 1994;9:139-143.

Cohen H, Bar-haim N, Kotler M. Acute inositol induces anxiety in rats. Biol Psychiatry 1996;40:426-427.

Cohen H, Kotler M, Kaplan Z, Matar MA, Kofman O, Belmaker RH. Inositol has behavioral effects with adaptation after chronic administration. J Neural Transm 1997;104:299-305.

Colodny L, Hoffman RL. Inositol – Clinical applications for exogenous use. Altern Med Review 1998;3:432-447.

Cooper JR, Bloom FE, Roth RH. The Biochemical Basis of Neuropharmacology, 8th Edition. Oxford University Press: Oxford, UK. 2003.

Coppen A. The biochemistry of affective disorders. Br J Psychiatry 1967;113:1237-1264.

Coupland NJ, Ogilvie CJ, Hegadoren KM, Seres P, Hanstock CC, Allen PS. Decreased prefrontal myo-inositol in major depressive disorder. Biol Psychiatry 2005;57:1526-1534.

Cowan B. Nuclear Magnetic Resonance and Relaxation. Cambridge University Press: Cambridge, UK. 1997.

Crandell DL. The adrenergic mechanism. Clin Anesth 1963;52:35-52.

Dalvit C, Wright PE. Assignment of resonances in the ^{1}H nuclear magnetic resonance spectrum of the carbon monoxide complex of human hemoglobin alpha-chains. J Mol Biol 1987;194:329-339.

Daniel ED, Cheng L, Maycox PR, Mudge AW. The common inositol-reversible effect of mood stabilizers on neurons does not involve GSK3 inhibition, myo-inositol-1-phosphate synthase or the sodium-dependent myo-inositol transporters. Mol Cell Neurosci 2006: Online ahead of print.

Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M, McCracken J. Decreased anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. Neuropsychopharmacol 2001;24:359-369.

Davanzo P, Yue K, Thomas MA, Belin T, Mintz J, Venkatraman TN, and others. Proton magnetic resonance spectroscopy of bipolar disorder versus intermittent explosive disorder in children and adolescents. Am J Psychiatry 2003;160:1442-1452.

Davis JM, Janicak PG, Hogan DM. Mood stabilizers in the prevention of recurrent affective disorders: A meta-analysis. Acta Psychiatrica Scandinavia 1999;100:406-417.

Deicken RF, Eliaz Y, Feiwell R, Schuff N. Increased thalamic N-acetylaspartate in male patients with familial bipolar I disorder. Psychiatry Res Neuroimag 2001;106:35-45.

del Rio E, Shinomura T, van der Kaay J, Nicholls DG, Downes CP. Disruption by lithium of phosphoinositide signalling in cerebellar granule cells in primary culture. J Neurochem 1998;70:1662-1669.

Derivan A, Entsuah AR, Kikta D. Venlafaxine: Measuring the onset of antidepressant action. Psychopharmacol Bull 1995;31:439-447.

Desmond P, Tress B. Magnetic Resonance Imaging. In: Neuroimaging and the Psychiatry of Late Life. Ames D, Chiu E (eds.). Cambridge University Press: London, UK. 1997.

Deverteuil RL, Lehmann HE. Therapeutic trail of iproniazid (Marsilid) in depressed and apathetic patients. Can Med Assoc J 1958;78:131-133.

Dick P. Therapeutic action of a monoamine oxidase inhibitor, Marsilid (iproniazid), on depressive states. Schweiz Med Wochenschr 1959;89:1288-1291.

Dierick M, Ravizza L, Realini R, Martin A. A double-blind comparison of venlafaxine and fluoxetine for treatment of major depression in outpatients. Prog Neuropsychopharmacol Biol Psychiatry 1996;20:57-71.

Dixon JF, Hokin LE. The antibipolar drug valproate mimics lithium in stimulating glutamate release and inositol 1,4,5-trisphosphate accumulation in brain cortex slices but not accumulation of inositol monophosphates and bisphosphates. Proc Natl Acad Sci USA 1997;94:4757-4760.

Dohn DR, Quebbemann AJ, Borch RF, Anders MW. Enzymatic reaction of chlorotrifluoroethene with glutathione: 19F NMR evidence for stereochemical control of the reaction. Biochemistry 1985;24:5137-5143.

Doisy EA Jr. Inositol. In: The Encyclopedia of Biochemistry. Williams RJ, Lansford EM Jr (eds.). Reinhold Publishing Corporation: New York, NY. 1967.

Dunner DL, Dwyer T, Fieve RR. Depressive symptoms in patients with unipolar and bipolar affective disorder. Compr Psychiatry 1976;17:447-451.

Dziedzicka-Wasylewska M, Willner P, Papp M. Changes in dopamine receptor mRNA expression following chronic mild stress and chronic antidepressant treatment. Behav Pharmacol 1997;8:607-618.

Einat H, Elkabaz-Shwortz Z, Cohen H, Kofman O, Belmaker RH. Chronic epi-inositol has an anxiolytic-like effect in the plus-maze model in rats. Int J Neuropsychopharmcol 1998;1:31-34.

Einat H, Belmaker RH, Kopilov M, Klein E, Gazawi H, Ben-Shachar D. Rat brain monoamines after acute and chronic myo-inositol treatment. Eur Neuropsychopharmacol 1999b;10:27-30.

Einat H, Karbovski H, Korik J, Tsalah D, Belmaker RH. Inositol reduces depressive-like behaviors in two different animal models of depression. Psychopharmacology 1999a;144:158-162.

Einat H, Belmaker RH. The effects of inositol treatment in animal models of psychiatric disorders. J Affect Disord 2001;62:113-121.

Einat H, Clenet F, Shaldubina A, Belmaker RH, Bourin M. The antidepressant activity of inositol in the forced swim test involves 5-HT(2) receptors. Behav Brain Res 2001;118:77-83.

Einat H, Belmaker RH, Zangen A, Overstreet DH, Yadid G. Chronic inositol treatment reduces depression-like immobility of Flinders Sensitive Line rats in the forced swim test. Depress Anxiety 2002;15:148-151.

Evins AE, Demopulos C, Yovel I, Culhane M, Ogutha J, Grandin LD, Nierenberg AA, Sachs GS. Inositol augmentation of lithium or valproate for bipolar depression. Bipolar Disord 2006;8:168-174.

Farmer A, McGuffin P. The classification of the depressions. Contemporary confusion revisisted. Br J Psychiatry 1989;155:437-443.

Farrar TC. Principles of pulse NMR spectroscopy. In: NMR: Principles and Applications to Biomedical Research. Pettegrew JW (ed.). Springer-Verlag: New York, NY. 1990.

Feighner JP. Mechanism of action of antidepressant medications. J Clin Psychiatry 1999;60(Suppl 4):4-11.

Fieve RR. Lithium therapy at the millennium: A revolutionary drug used for 50 years faces competing options and possible demise. Bipolar Disord 1999;1:67-70.

Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in the nervous system: An update. J Neurochem 1992;58:18-38.

Fisher SK, Novak JE, Agranoff BW. Inositol and higher inositol phosphates in neural tissue: Homeostasis, metabolism and functional significance. J Neurochem 2002;82:736-754.

Fogarty F, Russell JM, Newman SC, Bland RC. Mania. Acta Psychiatr Scand 1994;Suppl 376:16-23.

Frey R, Metzler D, Fischer P, Heiden A, Scharfetter J, Moser E, Kasper S. Myo-inositol in depressive and healthy subjects determined by frontal ^{1}H-magnetic resonance spectroscopy at 1.5 tesla. J Psychiatr Res 1998;32:411-420.

Frye M, Ketter T, Kimbrell T, Dunn RT, Speer AM, Osuch EA, Luckenbaugh DA, Cora-Ocatelli G, Leverich GS, Post RM. A placebo-controlled study of lamotrigine and gabapentin monotherapy in refractory mood disorders. J Clin Psychopharmacol 2000;20:607-614.

Fux M, Levine J, Aviv A, Belmaker RH. Inositol treatment of obsessive-compulsive disorder. Am J Psychiatry 1996;153:1219-1221.

Gajwani P, Forsthoff A, Muzina D, Amann B, Gao K, Elhaj O, Calabrese JR, Grunze H. Antiepileptic drugs in mood-disordered patients. Epilepsia 2005;46(Suppl 4):38-44.

Gani D, Downes CP, Batty I, Bramham J. Lithium and myo-inositol homeostasis. Biochimica et Biophysica Acta 1993;1177:253-269.

Gelder M, Mayou R, Cowen P. Mood disorders. In: Shorter Oxford Textbook of Psychiatry. Oxford University Press: Oxford, UK. 2001.

Gershon E, Hamovit J, Guroff JJ, Dibble E, Leckman JF, Sceery W, Targum SD, Nurnberger JI Jr, Goldin LR, Bunney WE Jr. A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. Arch Gen Psychiatry 1982;39:1157-1167.

Gijsman HJ, Geddes JR, Rendell JM, Nolen WA, Goodwin GM. Antidepressants for bipolar depression: A systematic review of randomized, controlled trials. Am J Psychiatry 2004;161:1537-1547.

Goldsmith DR, Wagstaff AJ, Ibbotson T, Perry CM. Spotlight on lamotrigine in bipolar disorder. CNS Drugs 2004;18:63-67.

Goldsmith DR, Wagstaff AJ, Ibbotson T, Perry CM. Lamotrigine: A review of its use in bipolar disorder. Drugs 2003;63:2029-2050.

Goodwin FK, Jamison KR. Manic Depressive Illness. Oxford University Press: New York, NY. 1990.

Gorman JM, Kent JM. SSRIs and SNRIs: Broad spectrum of efficacy beyond major depression. J Clin Psychiatry 1999;60(Suppl 4):33-38.

Gould TD, Quiroz JA, Singh J, Zarate CA Jr, Manji HK: Emerging experimental therapeutics for bipolar disorder: Insights from the molecular and cellular actions of current mood stabilizers. Mol Psychiatry 2004;9:734-755.

Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed 2000;13:129-153.

Greil W, Steber R, van Calker D. The agonist-stimulated accumulation of inositol phosphates is attenuated in neutrophils from male patients under chronic lithium therapy. Biol Psychiatry 1991;30:443-451.

Guelfi JD, White C, Hackett D, Guichoux JY, Magni G. Effectiveness of venlafaxine in patients hospitalized for major depression and melancholia. J Clin Psychiatry 1995;56:450-458.

Gunther H. NMR Spectroscopy: Basic Principles, Concepts, and Applications in Chemistry, 2nd Edition. John Wiley & Sons: New York, NY. 1995.

Hahn CG, Umapathy, Wang HY, Koneru R, Levinson DF, Friedman E. Lithium and valproic acid treatment reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. J Psychiatr Res 2005;39(4):355-363.

Hall LD. Nuclear Magnetic Resonance. Adv Carbohydr Chem 1964;19:51-93.

Hamakawa H, Kato T, Shioiri T, Inubushi T, Kato, N. Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. Psychol Med 1999;29:639-644.

Heun R, Maier W. The distinction of bipolar II disorder from bipolar I and recurrent unipolar depression: Results of a controlled family study. Acta Psychiatr Scand 1993;87:279-284.

Hirschfeld RMA. Bipolar depression: The real challenge. Eur Neuropsychopharmacol 2004;14:S83-S88.

Holub BJ. The nutritional significance, metabolism, and function of *myo*-inositol and phosphatidylinositol in health and disease. Adv Nutr Res 1982;4:107-141.

Isaacks RE, Bender AS, Kim CY, Prieto NM, Norenberg MD. Osmotic regulation of *myo*-inositol uptake in primary astrocyte cultures. Neurochem Res 1994;19:331-338.

Ishida T, In Y, Shibata M, Doi M, Inoue M, Yanagisawa I. On the structure-activity relationship of histamine H2-receptor antagonists based on the X-ray crystal structures and 1H-NMR spectra of amidine derivatives. Mol Pharmacol 1987;31:410-416.

Jacquart D. Les medecins et les secrets de la bile noire. L'Histoire 2004;285:44-47.

Jenkinson S, Patel N, Nahorski SR, Challiss RA. Comparative effects of lithium on the phosphoinositide cycle in rat cerebral cortex, hippocampus, and striatum. J Neurochem 1993;61:1082-1090.

Jouanna J. Hippocrates. Translated by: DeBevoise MB. The Johns Hopkins University Press: Baltimore, MD. 1999.

Ju S, Greenberg ML. Valproate disrupts regulation of inositol responsive genes and alters regulation of phospholipids biosynthesis. Mol Microbiol 2003;49:1595-1603.

Kaplan Z, Amir M, Swartz M, Levine J. Inositol treatment of post-traumatic stress disorder. Anxiety 1996;2:51-52.

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002;109:143-148.

Kasper S. Bipolar disorder: A new field for rational polypharmacy. In: Maj M, Akiskal HS, Lopez-Ibor JJ, Sartorius N (eds.). WPA Series, Evidence and Experience in Psychiary, Volume 5: Bipolar Disorder. John Wiley & Sons: London, UK. 2002.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded ^{31}P-MRS. Psychol Med 1995;25:557-566.

Kato T, Takahashi S, Shioiri T, Inubushi T. Brain phosphorus metabolism in depressive disorders detected by phosphorus-31 magnetic resonance spectroscopy. J Affect Disord 1992;26:223-230.

Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. J Affect Disord 1994;31:125-133.

Keck PE Jr., McElroy SL, Richtand N, Tohen M. What makes a drug a primary mood stabilizer? Mol Psychiatry 2002;7:S8-S14.

Kelsoe JR. Arguments for the genetic basis of the bipolar spectrum. J Affect Disord 2003;73:183-197.

Kendell RE. The classification of depression: A review of contemporary confusion. Br J Psychiatry 1976;129:15-28.

Kendler KS, Pedersen N, Johnson L, Neale MC, Mathe AA. A pilot Swedish twin study of affective illness, including hospital- and population-ascertained subsamples. Arch Gen Psychiatry 1993;50:699-700.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, and others. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in United States. Results from the national comorbidity survey. Arch Gen Psychiatry 1994;51:8-19.

Kim H, McGrath BM, Silverstone PH. A review of the possible relevance of inositol and the phosphatidylinositol second messenger system (PI-cycle) to psychiatric disorders – focus on magnetic resonance spectroscopy (MRS) studies. Hum Psychopharmacol Clin Exp 2005;20:309-326.

Kim H, Thompson RB, Hanstock CC, Allen PS. Variability of metabolite yield using STEAM or PRESS sequences in vivo at 3.0 T, illustrated with myo-inositol. Magn Reson Med 2005;53:760-769.

Kofman O, Einat H, Cohen H, Tenne H, Shoshana C. The anxiolytic effect of chronic inositol depends on the baseline level of anxiety. J Neural Transm 2000;107:241-253.

Kofman O, Agam G, Shapiro J, Spencer A. Chronic dietary inositol enhances locomotor activity and brain inositol levels in rats. Psychopharmacology 1998;139:239-242.

Kuhn R. Uber die behandlung depressiver zustande mit einem Iminodibenzylderivat. Schweiz Med Wochenschr 1957;87:1135-1140.

Kuhn R. The treatment of depressive states with G 22355 (imipramine hydrochloride). Am J Psychiatry 1958;115:459-464.

Kusumakar V, Yatham LN, Haslam DR, Parikh SV, Matte R, Silverstone PH, Sharma V. Treatment of mania, mixed states, and rapid cycling. Can J Psychiatry 1997;42:79S-86S.

Lammers CH, Diaz J, Schwartz JC, Sokoloff P. Selective increase of dopamine D3 receptor gene expression as a common effect of chronic antidepressant treatments. Mol Psychiatry 2000;5:378-388.

Lehmann HE, Cahn CH, De Vereuil RL. The treatment of depressive conditions with imipramine (G 22355). Can Psychiatr Assoc J 1958;3:155-164.

Levine J. Controlled trials of inositol in psychiatry. Eur Neuropsychopharmacol 1997;7:147-155.

Levine J, Barak Y, Gonzalves M, Szor H, Elizur A, Kofman O, Belmaker RH. Double-blind, controlled trial of inositol treatment of depression. Am J Psychiatry 1995;152:792-794.

Levine J, Mishori A, Susnosky M, Martin M, Belmaker RH. Combination of inositol and serotonin reuptake inhibitors in the treatment of depression. Biol Psychiatry 1999;45:270-273.

Lewis J, Winokur G. The induction of mania: A natural history study with controls. Arch Gen Psychiatry 1982;39:303-306.

Li PP, Sibony D, Green MA, Warsh JJ. Lithium modulation of phosphoinositide signaling system in rat cortex: selective effect on phorbol ester binding. J Neurochem 1993;61:1722-1730.

Lloyd KG, Thuret F, Pilc A. Upregulation of gamma-aminobutyric acid (GABA) B binding sites in rat frontal cortex: A common action of repeated administration of different classes of antidepressants and electroshock. J Pharmacol Exp Ther 1985;235:191-199.

Lubrich B, Patishi Y, Kofman O, Agam G, Berger M, Belmaker RH, van Calker D. Lithium-induced inositol depletion in rat brain after chronic treatment is restricted to the hypothalamus. Mol Psychiatry 1997;2:407-412.

Macritchie K, Geddes J, Scott J, Haslam D, de Lima M, Goodwin G. Valproate for acute mood episodes in bipolar disorder. Cochrane Database Syst Rev 2003:1:CD004052.

Macomber RS. A Complete Introduction to Modern NMR Spectroscopy. John Wiley & Sons: New York, NY. 1998.

Maj M, Pirozzi R, Magliano L, Bartoli L. The prognostic significance of "switching" in patients with bipolar disorder: A 10-year prospective follow-up study. Am J Psychiatry 2002;159:1711-1717.

Manji HK, Lenox RH. Long-term action of lithium: A role for transcriptional and posttranscriptional factors regulated by protein kinase C. Synapse 1994;16:11-28.

Marneros A, Angst J (eds.) Bipolar Disorders: 100 Years after Manic Depressive Insanity. Kluwer: Dordrecht. 2000.

Marneros A, Brieger P. Prognosis of bipolar disorder: A review. In: Maj M, Akiskal HS, Lopez-Ibor JJ, Sartorius N (eds.). WPA Series, Evidence and Experience in Psychiatry, Volume 5: Bipolar Disorder. John Wiley & Sons: London, UK. 2002.

Merikangas KR, Chakravarti A, Moldin SO, Araj H, Blangero JC, Burmeister M, Crabbe J Jr, Depaulo JR Jr, Foulks E, Freimer NB, Koretz DS, Lichtenstein W, Mignot E, Reiss AL, Risch NJ, Takahashi JS. Future of genetics of mood disorders research. Biol Psychiatry 2002;52:457-477.

McElroy SL, Keck PE Jr., Strakowski SM. Mania, psychosis and antipsychotics. J Clin Psychiatry 1996;57:14-26.

McGrath BM. Towards a comprehensive understanding of bipolar disorder: In vivo MRS investigation of the phosphatidylinositol cycle. McGill J Med 2004;8:40-49.

McGrath BM, Wessels PH, Bell EC, Ulrich M, Silverstone PH. Neurobiological findings in bipolar II disorder compared with findings in bipolar I disorder. Can J Psychiatry 2004;49:794-801.

Montgomery SA, McAuley R, Rani SJ, Roy D, Montogmery DB. A double blind comparison of zimelidine and amitriptyline in endogenous depression. Acta Psychiatr Scand 1981:63(Suppl 290):314-327.

Moore CM, Breeze JL, Kukes TJ, Rose SL, Dager SR, Cohen BM, Renshaw PF. Effect of *myo*-Inositol ingestion on human brain *myo*-Inositol levels: a proton magnetic resonance spectroscopic imaging study. Biol Psychiatry 1999;45:1197-1202.

Moore CM, Breeze JL, Gruber SA, Babb SM, Frederick B, Villafuerte RA, Stoll AL, Hennen J, Yurgelun-Todd DA, Cohen BM, Renshaw PF. Choline, myo-inositol and mood in bipolar disorder: A proton magnetic resonance spectroscopy imaging study of the anterior cingulate cortex. Bipolar Disord 2000;2:207-216.

Murray M, Greenberg ML. Expression of yeast INM1 encoding inositol monophosphatase is regulated by inositol, carbon source and growth stage and is decreased by lithium and valproate. Mol Microbiol 2000;36:651-661.

Murray CJL, Lopez AD. (eds.). The global burden of disease: A comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Global Burden of Disease and Injury Series, Vol. 1. Harvard University Press: Cambridge, UK. 1996.

Naccarato WF, Ray RE, Wells WW. Biosynthesis of myo-inositol in rat mammary gland. Isolation and properties of the enzymes. Arch Biochem Biophys 1974;164:194-201.

Nestler EJ, Hyman SE, Malenka RC. Molecular Neuropharmacology: A Foundation for Clinical Neuroscience. McGraw-Hill: New York, USA. 2001.

Nierenberg AA, Ostacher MJ, Calabrese JR, Ketter TA, Marangell LB, Miklowitz DJ, Miyahara S, Bauer MS, Thase ME, Wisniewski SR, Sachs GS. Treatment-resistent bipolar depression: A STEP-BP equipoise randomized effectiveness trial of antidepressant augmentation with lamotrigine, inositol, or risperidone. Am J Psychiatry 2006;163:210-216.

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH. Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 2000;880:84-91.

O'Donnell T, Rotzinger S, Ulrich M, Hanstock CC, Nakashima TT, Silverstone PH. Effects of chronic lithium and sodium valproate on concentrations of brain amino acids. Eur Neuropsychopharmacol 2003;13:220-227.

Oxenkrug GF, Lapin IP. Effect of dimethyl and monomethyl tricyclic antidepressants on central 5-hydroxytryptamine processes in the frog. J Pharm Pharmacol 1971;23:971-972.

Papp M, Moryl E. Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. Eur J Pharmacol 1994;263:1-7.

Paraskevas GP, Triantafyllou NI, Kapaki E, Limpitaki G, Petropoulou O, Vassilopoulos D. Add-on lamotrigine treatment and plasma glutamate levels in epilepsy: Relation to treatment response. Epilepsy Res 2006;Epub.

Patishi Y, Lubrich B, Berger M, Kofman O, van Calher D, Belmaker RH. Differential uptake of myo-inositol in vivo into rat brain areas. Eur Neuropsychopharmacol 1996;6:73-75.

Petroff OA, Spencer DD, Alger JR. High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. Neurology 1989;39:1197-1202.

Pettegrew JW, Panchalingam K, Levine J, McClure RJ, Gershon S, Yao JK: Chronic myo-inositol increases rat brain phosphatidylethanolamine plasmalogen. Biol Psychiatry 2001;49:444-453.

Petty F. GABA and mood disorders: A brief review and hypothesis. J Affect Disord 1995;34(4):275-281.

Pfeuffer J, Tkac I, Provencher SW, Gruetter R. Toward an in vivo neurochemical profile: Quantification of 18 metabolites in short-echo-time 1H NMR spectra of the rat brain. J Magn Reson 1999;141:104-120.

Pincus HA, Tanielian TL, Marcus SC. Prescribing trends in psychotropic medications: Primary care, psychiatry, and other medical specialities. JAMA 1998;279:526-531.

Pleasure H. Psychiatric and neurological side-effects of isoniazid and iproniazid. AMA Arch Neurol Psychiatry 1954;72:313-320.

Pletscher A. The discovery of antidepressants: A winding path. Experientia 1991;47:4-8.

Popik P, Krawczyk M, Golembiowska K, Nowak G, Janowsky A, Skolnick P, Lippa A, Basile AS. Pharmacological profile of the "Triple" monoamine neurotransmitter uptake inhibitor, DOV 102,677. Cell Mol Neurobiol 2006, Epub ahead of print.

Post RM, Weiss SRB, Clark M, Chuang D-M, Hough C, Li H. Lithium, carbamazepine, and valproate in affective illness. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Prien RF, Klett CJ, Caffey EM. Lithium carbonate and imipramine in prevention of affective episodes. Arch Gen Psychiatry 1973;29:420-425.

Regier DA, Boyd JH, Burke JD Jr, Rae DS, Myers JK, Kramer M, Robins LN, George LK, Karno M, Locke BZ. One-month prevalence of mental disorders in the United States. Based on five epidemiologic catchment area sites. Arch Gen Psychiatry 1988;45:977-986.

Ross BD. Biochemical considerations in ^{1}H spectroscopy. Glutamate and glutamine; *myo*-inositol and related metabolites. NMR Biomed 1991;4:59-63.

Sadock BJ, Sadock VA. Kaplan & Sadock's Synopsis of Psychiatry, 9th Edition. Lippincott Williams & Wilkins: Philadelphia, PA. 2003.

Salibi N, Brown MA. Clinical MR Spectroscopy: First Principles. Wiley-Liss: New York, NY. 1998.

Sanders JKM, Hunter BK. Modern NMR Spectroscopy: A Guide for Chemists, 2nd Edition. Oxford University Press: Oxford, UK. 1997.

Saunders JC, Radinger N, Rochlin D, Kline NS. Treatment of depressed and regressed patients with iproniazid and reserpine. Dis Nerv Syst 1959;20:31-39.

Schaffer A, Cairney J, Cheung A, Veldhuizen S, Levitt A. Community survey of bipolar disorder in Canada: Lifetime prevalence and illness characteristics. Can J Psychiatry 2006;51:9-16.

Schildkraut JJ. The catecholamine hypothesis of affective disorders: A review of supporting evidence. Am J Psychiatry 1965;122:509-522.

Sharma R, Venkatasubramanian PN, Barany M, Davis JM. Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizoph Res 1992;8:43-49.

Sherman WR, Munsell LY, Gish BG, Honchar MP. Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney, and testis. J Neurochem 1985;44:798-807.

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE. Reduced frontal cortex inositol levels in postmortem brain of suicide victims and patients with bipolar disorder. Am J Psychiatry 1997;154:1148-1150.

Silverstone PH, McGrath BM, Kim H. Bipolar disorder and myo-inositol: A review of the magnetic resonance spectroscopy findings. Bipolar Disord 2005b;7:1-10.

Silverstone PH, McGrath BM, Wessels PH, Bell EC, Ulrich M. Current pathophysiological findings in bipolar disorder and in its subtypes. Curr Psychiatry Rev 2005a;1:75-101.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Human Psychopharmacol 2002;17:321-327.

Simpson SG, DePaulo JR. Fluoxetine treatment of bipolar II depression. J Clin Psychopharmacol 1991;11:52-54.

Simpson SG, McMahon FJ, McInnis MG, Edwin D, Folstein SE, DePaulo JR. Diagnostic reliability of bipolar II disorder. Arch Gen Psychiaty 2002;59:736-740.

Slattery DA, Hudson AL, Nutt DJ. Invited review: The evolution of antidepressant mechanisms. Fundamental Clin Pharmacol 2004;18:1-21.

Spaner D, Bland RC, Newman SC. Major Depressive Disorder. Acta Psychiatr Scand 1994;Suppl 376:7-15.

Spector R. Myo-inositol transport through the blood-brain barrier. Neurochem Res 1988;13:785-787.

Stahl SM, Grady MM, Moret C, Briley M. SNRIs: Their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. CNS Spectr 2005;10:732-747.

Steinberg R, Alonso R, Griebel G, Bert L, Jung M, Oury-Donat F, Poncelet M, Gueudet C, Desvignes C, Le Fur G, Soubrie P. Selective blockade of neurokinin-2 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. J Pharmacol Exp Ther 2001;299:449-458.

Sun GY, Navidi M, Yoa FG, Lin TN, Orth OE, Stubbs EB Jr, MacQuarrie RA. Lithium effects on inositol phospholipids and inositol phosphates: Evaluation of an in vivo model for assessing polyphosphoinositide turnover in brain. J Neurochem 1992;58:290-297.

Szadoczky E, Papp Z, Vitrai J, Rihmer Z, Zuredi J. The prevalence of major depressive and bipolar disorders in Hungary. Results from a national epidemiologic survey. J Affect Disord 1998;50:153-162.

Szegedi A. No delay in the onset of improvement once antidepressants are on board. Biol Psychiatry 2006;59(8, Suppl 1):17.

Tarasow E, Panasiuk A, Siergiejczyk L, Orzechowska-Bobkiewicz A, Lewszuk A, Walecki J, Prokopowicz D. MR and ^{1}H MR spectroscopy of the brain in patients with liver cirrhosis and early stages of hepatic encephalopathy. Hepatogastroenterol 2003;50:2149-2153.

Thase ME, Shelton RC, Khan A. Treatment with venlafaxine extended release after SSRI nonresponse or intolerance: A randomized comparison of standard- and higher-dosing strategies. J Clin Psychopharmacol 2006;26:250-258.

Thomas P. The many forms of bipolar disorder: A modern look at an old illness. J Affect Disord 2004;79:S3-S8.

Thurston JH, Sherman WR, Hauhart RE, Kloepper RF. Myo-inositol: A newly identified non-nitrogenous osmoregulatory molecule in mammalian brain. Pediatr Res 1989;26:482-485.

Tohen M, Greil W, Calabrese JR, Sachs GS, Yatham LN, Oerlinghausen BM, Koukopoulos A, Cassano GB, Grunze H, Licht RW, Dell'Osso L, Evans AR, Risser R, Baker RW, Crane H, Dossenbach MR, Bowden CL. Olanzapine versus lithium in the maintenance treatment of bipolar disorder: A 12-month, randomized, double-blind, controlled clinical trial. Am J Psychiatry 2005;167:1281-1290.

Tohen M, Ketter TA, Zarate CA, Suppes T, Frye M, Altshuler L, Zajecka J, Schuh LM, Risser RC, Brown E, Baker RW. Olanzapine versus divalproex sodium for the treatment of acute mania and maintenance of remission: A 47-week study. Am J Psychiatry 2003;160:1263-1271.

Tohen M, Tollefson GD. Atypical antipsychotic agents in mania: Clinical studies. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Tomb DA. Psychiatry, 6th Edition. Lippincott Williams & Wilkins: Philadelphia, PN. 1999.

Tremblay P, Blier P. Catecholaminergic strategies for the treatment of major depression. Curr Drug Targets 2006;7:149-158.

Ustun TB, Chisholm D. Global burden of disease – Study for psychiatric disorders. Psychiatrische Prax 2001;28:7-11.

Vaden DL, Ding D, Peterson B, Greenberg ML. Lithium and valproate decrease inositol mass and increase expression of the yeast *INO1* and *INO2* genes for inositol biosynthesis. J Biol Chem 2001;276:15466-15471.

Vadnal R, Parthasarathy R. Myo-inositol monophosphatase: Diverse effects of lithium, carbamazepine, and valproate. Neuropsychopharmacol 1995;12:277-285.

Vandal R. Role of inositol in the treatment of psychiatric disorders. CNS Drugs 1997;7:6-16.

Wasserman MJ, Corson TW, Sibony D, Cooke RG, Parikh SV, Pennefather PS, Li PP, Warsh JJ. Chronic lithium treatment attenuates intracellular calcium mobilization. Neuropsychopharmacol 2004;29:759-769.

Watson DG, Lenox RH. Chronic lithium-induced down-regulation of MARCKS in immortalized hippocampal cells: Potentiation by muscarinic receptor activation. J Neurochem 1996;67:767-777.

Wehr TA, Goodwin FK. Rapid cycling in manic-depressives induced by tricyclic antidepressants. Arch Gen Psychiatry 1979;36:555-559.

Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, Joyce PR, Karam EG, Lee CK, Lellouch J, Lepine JP, Newman SC, Rubio-Stipec M, Wells JE, Wickramaratne PJ, Wittchen H, Yeh EK. Cross-national epidemiology of major depression and bipolar disorder. JAMA 1990;276:293-299.

Williams RS, Cheng L, Mudge AW, Harwood AJ. A common mechanism of action for three mood-stabilizing drugs. Nature 2002;417:292-295.

Williams S. Cerebral amino acids studies by nuclear magnetic resonance spectroscopy in vivo. Progr Nucl Mag Res Spectr 1999;34:301-326.

Wolfson M, Bersudsky Y, Zinger E, Simkin M, Belmaker RH, Hertz L. Chronic treatment of human astrocytoma cells with lithium, carbamazepine or valproic acid decreases inositol uptake at high inositol concentrations but increases it at low inositol concentrations. Brain Res 2000;855:158-161.

Yatham LN, Kennedy SH, O'Donovan C, Parikh S, MacQueen G, McIntyre R, Sharma V, Silverstone P, Alda M, Baruch P, Beaulieu S, Daigneault A, Milev R, Young T, Ravindran A, Schaffer A, Connolly M, Gorman CP. Canadian Network for Mood and Anxiety Treatments (CANMAT) guidelines for the management of patients with bipolar disorder: Consensus and controversies. Bipolar Disord 2005;7(Suppl 3):5-69.

York JD, Guo S, Odom AR, Spiegelberg BD, Stolz LE. An expanded view of inositol signaling. Adv Enzyme Regul 2001;41:57-71.

Chapter 2. Literature Review

(Parts of this chapter have been published in *Bipolar Disorders* or *Human Psychopharmacology* – see Silverstone et al. 2005; Kim et al. 2005)

This chapter reviews recent studies that have added to our understanding of the pathophysiology of BPD and UPD. Particularly, this chapter will review those ^{1}H-MRS studies that have investigated PI-cycle functioning, via measurements of *myo*-inositol concentration *in vivo,* in order to determine and better characterize the underlying differences that exist between BPD, UPD and healthy controls (HC). For completeness, findings from the ^{31}P-MRS literature have also been included. These studies report on measurements of the PME peak, which contains contributions from the inositol monophosphates, the metabolic precursors to *myo*-inositol in the PI-cycle. In addition, where evidence exists, possible differences in *myo*-inositol and PME between patients with BPD-I, BPD-II and UPD will be examined.

2.1 MRS findings in Bipolar and Unipolar Disorders

Much of the work investigating the role of *myo*-inositol in psychiatric disorders has focused on BPD. This followed earlier findings suggesting that lithium may be clinically effective via its attenuation of *myo*-inositol production. This may follow from its effect as an uncompetitive inhibitor of IMPase (Allison et al. 1971, 1976; Berridge et al. 1982), the enzyme responsible for breaking down inositol monophosphates into *myo*-inositol (Figure 1.2). This uncompetitive inhibition implies the involvement of an overactive PI-cycle turnover in bipolar symptomatology, detectable by increased levels of PI-cycle constituents, including *myo*-inositol and PME.

2.1.1 Bipolar Depression

Table 2.1 summarizes the MRS findings in bipolar depression to date. In a study of unmedicated BPD and UPD patients, Frey and associates (1998) reported reduced *myo*-inositol concentrations in the frontal lobes of patients compared to HC, although this finding was significant only when the groups were paired by age. In a well-conducted study by Moore and colleagues (1999) in which absolute metabolite concentrations were measured, a reduction of *myo*-inositol concentrations was reported in the frontal lobe of medicated depressed BPD patients, but not in the occipital, parietal or temporal regions, following both acute (5-7 days) and chronic (3-4 weeks) lithium treatment. In a separate study, no differences in *myo*-inositol concentrations were reported for the anterior cingulate cortex between depressed BPD patients and HC (Moore et al. 2000) and in the dorsolateral prefrontal cortex (DLPFC) of HC following lithium treatment (Brambilla et al. 2004). Recent studies failed to find any differences between white matter *myo*-inositol concentrations between drug-free depressed and mixed-mood BPD patients and HC (Dager et al. 2004), and in grey matter *myo*-inositol among lithium-treated BPD patients, but not valproate-treated BPD patients (Friedman et al. 2004), relative to HC. In a series of studies, Kato and associates (1992, 1994b, 1995) also reported higher PME concentrations in the frontal lobe of lithium-treated depressed BPD patients compared to both euthymic patients and HC.

Table 2.1: MRS Findings for *myo*-Inositol and Phosphomonoesters in Depressed Bipolar Patients.

Study	Technique	N[a]	Findings	Notes
Kato et al. 1992	1.5T ^{31}P-MRS DRESS	10	- ↑ frontal lobe PME in depressed BPD-II patients compared to euthymic BPD-II patients.	Various medications
Kato et al. 1994b	1.5T ^{31}P-MRS DRESS	29	- ↑ frontal lobe PME in depressed BPD-II patients compared to HC.	Various Medications
Kato et al. 1995	1.5T ^{31}P-MRS Phase-encoded	25	- ↑ left frontal lobe PME in depressed BPD patients compared to HC.	Lithium
Frey et al. 1998	1.5T ^{1}H-MRS STEAM	22	- ↓ right frontal lobe m-Ino in older depressed BPD and UPD patients compared to HC.	Anti-depressants
Moore et al. 1999	1.5T ^{1}H-MRS STEAM	12	- ↓ right frontal lobe m-Ino in depressed BPD-I patients compared to HC. - ↔ temporal, parietal and occipital lobe m-Ino between depressed BPD-I patients and HC.	Post-Lithium
Moore et al. 2000	1.5T ^{1}H-MRS STEAM	9	- ↔ anterior cingulated cortex m-Ino between depressed BPD I patients and HC, irrespective of antidepressant treatment.	Various Medications
Friedman et al. 2004	1.5T ^{1}H-MRS PEPSI	21	- ↑ grey matter m-Ino in lithium-treated depressed or mixed-mood BPD patients compared to HC. - ↔ grey matter m-Ino between lithium and valproate-treated depressed or mixed-mood BPD patients. - ↔ grey matter m-Ino between valproate-treated depressed or mixed-mood BPD patients and healthy controls.	Post-Lithium or Valproate
Dager et al. 2004	1.5T ^{1}H-MRS PEPSI	32	- ↔ regional m-Ino between depressed or mixed-mood BPD-I and BPD-II patients and HC. - ↔ white matter m-Ino between depressed or mixed-mood BPD-I and BPD-II patients and HC.	Drug-Free

[a] The sample size represents the number of patients (not controls) in each study.

2.1.2 Bipolar Mania and Hypomania

Table 2.2 summarizes the MRS findings in bipolar mania and hypomania to date. In a study examining patients before and after lithium treatment, there was a non-significant trend at baseline towards higher *myo*-inositol concentrations in the anterior cingulate cortex of manic BPD children compared to HC (Davanzo et al. 2001). Following 7 days lithium treatment, *myo*-inositol concentrations in the anterior cingulate cortex were significantly reduced in children with mania compared to the HC. In a follow-up study the same group reported statistically significant increased *myo*-inositol concentrations in the anterior cingulate cortex of manic BPD children compared to HC, thus confirming the trend observed in their early study (Davanzo et al. 2003).

Reduced *myo*-inositol was observed in the basal ganglia of 4 lithium-treated manic BPD patients, while no difference was observed for occipital cortex when compared to HC (Sharma 1992). In three studies one group found higher PME concentrations in the frontal lobe of lithium-treated manic and hypomanic BPD patients compared to euthymic BPD patients and HC (Kato et al. 1991, 1993, 1994b). However, this finding was not supported in a subsequent study carried out by the same group (Kato et al. 1995). A study of manic and mixed BPD patients, most of who were receiving sodium valproate in addition to other medications, found no differences in frontal lobe *myo*-inositol concentrations when compared to HC (Cecil et al. 2002).

Table 2.2: MRS Findings for *myo*-Inositol and Phosphomonoesters in Manic/Hypomanic Bipolar Patients.

Study	Technique	N[a]	Findings	Notes
Kato et al. 1991	1.5T ^{31}P-MRS DRESS	11	- ↑ frontal lobe PME in manic BPD-I patients compared to HC.	Lithium
Sharma et al. 1992	1.5T ^{1}H-MRS STEAM	4	- ↑ basal ganglia m-Ino in manic BPD-I patients compared to HC. - ↔ occipital lobe mIno between manic BPD-I patients and HC.	Lithium
Kato et al. 1993	1.5T ^{31}P-MRS DRESS	17	- ↑ frontal lobe PME in manic BPD-I patients compared to euthymic BPD-I patients.	Lithium
Kato et al. 1994b	1.5T ^{31}P-MRS DRESS	29	- ↑ frontal lobe PME in hypomanic BPD-II patients compared to HC.	Various Medications
Kato et al. 1995	1.5T ^{31}P-MRS Phase-encoded	25	- ↔ frontal lobe PME between manic BPD patients and HC.	Lithium
Davanzo et al. 2001	1.5T ^{1}H-MRS PRESS	11	- ↑ anterior cingulate cortex m-Ino in untreated manic and hypomanic BPD (I and II) patients compare to HC. - ↓ anterior cingulate cortex m-Ino in post-lithium manic and hypomanic BPD (I and II) patients compare to HC.	Children
Cecil et al. 2002	1.5T ^{1}H-MRS PRESS	17	- ↔ frontal lobe m-Ino between manic and mixed BPD-I patients and HC.	Various Medications
Davanzo et al. 2003	1.5T ^{1}H-MRS PRESS	10	- ↑ anterior cingulate cortex m-Ino in manic or mixed BPD-I patients compared to HC. - ↔ anterior cingulated cortex m-Ino between manic or mixed BPD-I patients and HC.	Drug-Free Children

[a] The sample size represents the number of patients (not controls) in each study.

2.1.3 Bipolar Euthymia

Table 2.3 summarizes the MRS findings in euthymic BPD patients to date. Most studies have suggested that euthymic patients, whether treated or not, appear to have normal *myo*-inositol and inositol monophosphates concentrations, although some studies have found these metabolites to be reduced. Thus, using ^{1}H-MRS, no differences in frontal lobe *myo*-inositol concentrations were observed between unmedicated euthymic BPD patients and HC (Winsberg et al. 2000). Another study found no difference in PME concentrations in the frontal lobe of unmedicated euthymic BPD patients when compared with HC (Kato et al. 1998). In contrast, however, two ^{31}P-MRS studies reported significantly lower PME concentrations in the frontal (Deicken et al. 1995a) and temporal (Deicken et al. 1995b) lobes of unmedicated, euthymic BPD patients compared to HC.

Among medicated euthymic BPD patients, three ^{1}H-MRS studies reported no changes in *myo*-inositol concentrations in treated BPD patients (Bruhn et al. 1993; Silverstone et al. 2002; Chang et al. 2003). Several studies utilizing ^{31}P MRS suggest that euthymic BPD patients on treatment have PME concentrations that are not significantly different from HC (Kato et al. 1994b, 1995; Murashita et al. 2000; Silverstone et al. 2002; Hamakawa et al. 2004), although other studies did find reduced concentrations (Kato et al. 1993, 1994a).

Table 2.3: MRS Findings for *myo*-Inositol and Phosphomonoesters in Euthymic Bipolar Patients.

Study	Technique	N[a]	Findings	Notes
Kato et al. 1992	1.5T ^{31}P-MRS DRESS	10	- ↓frontal lobe PME in euthymic BPD-II patients compared to HC.	Various medications
Kato et al. 1993	1.5T ^{31}P-MRS DRESS	17	- ↓frontal lobe PME in euthymic BPD-I patients compared to HC.	Lithium
Bruhn et al. 1993		7	- ↔temporal lobe mIno between euthymic BPD patients and HC.	Post-Lithium
Kato et al. 1994a	1.5T ^{31}P-MRS DRESS	40	- ↓frontal lobe PME in euthymic BPD-I patients compared euthymic BPD-II patients and HC.	Lithium
Kato et al. 1994b	1.5T ^{31}P-MRS DRESS	29	- ↓frontal lobe PME in euthymic BPD-I patients compared to BPD-II patients and HC.	Various Medications
Deicken et al. 1995a	2T ^{31}P-MRS Spin-echo	12	- ↓right and left frontal lobe PME and PDE in euthymic BPD patients compared to HC.	Drug-Free
Deicken et al. 1995b	2T ^{31}P-MRS STEAM	12	- ↓right and left temporal lobe PME in euthymic BPD patients compared to HC. - ↔right and left temporal lobe PDE between euthymic BPD patients and HC.	Drug-Free
Kato et al. 1995	1.5T ^{31}P-MRS Phase-encoded	25	- ↔frontal lobe PME between euthymic and manic BPD patients and HC.	Lithium
Winsberg et al. 2000	1.5T ^{1}H-MRS PRESS	20	- ↔right and left DLPFC m-Ino between euthymic BPD-I and BPD-II patients and HC.	Drug-Free
Murashita et al. 2000	1.5T ^{31}P-MRS	19	- ↔occipital lobe PME and PDE between euthymic BPD (I and II) patients and HC. - ↔occipital lobe PME and PDE between lithium-resistant and lithium-responsive euthymic BPD (I and II) patients.	Various Medications
Silverstone et al. 2002	3T ^{31}P-MRS PRESS	36	- ↔frontal lobe m-Ino and PME between euthymic BPD (I and II) patients and HC, irrespective of treatment.	Post-Lithium or Valproate
Chang et al. 2003	3T ^{1}H-MRS PRESS	15	- ↔DLPFC m-Ino between familial euthymic BPD-I patients and HC.	Children Various Medications
Hamakawa et al. 2004	1.5T ^{31}P-MRS DRESS	13	- ↔basal ganglia PME and PDE between euthymic BPD patients and HC.	Various medications

[a] The sample size represents the number of patients (not controls) in each study.

2.1.4 Unipolar Major Depressive

It is conceivable that the PI-cycle may be involved in some aspects of UPD, since platelets from UPD patients have been found to have increased inositol triphosphate binding sites (Dwivedi et al. 1998) as well as increased inositol monophospate (Mikuni et al. 1991; Pandey et al. 2001) and inositol triphosphate (Alvarez et al. 1999) concentrations. Moreover, treatment with antidepressants appears to normalize increased inositol triphosphate concentrations in responders only (Alvarez et al. 1999). While not as extensively studied as BPD, several MRS studies have investigated *myo*-inositol and PME, and these are summarized in Table 2.4.

In a study of unmedicated UPD patients, Kumar and associates (2002) reported increased *myo*-inositol concentrations in frontal white matter. Wyckoff and colleagues (2003) also reported increased *myo*-inositol concentrations in the left dorsolateral white matter of unmedicated late-life UPD patients. Moreover, a recent study correlated the increase in left DLPFC *myo*-inositol with increased cognitive function in HC but not in late-life UPD patients (Elderkin-Thompson et al. 2004). In contrast, in similar studies, no differences in frontal lobe *myo*-inositol concentrations were found between unmedicated UPD patients and HC (Gruber et al. 2003; Binesh et al. 2004). In a recent ^{1}H-MRS study, reduced dorsomedial prefrontal cortex (DMPFC) *myo*-inositol was found in 13 unmedicated UPD patients (Coupland et al. 2005), with similar findings reported in the anterior cingulate cortex as well (Rosenberg et al. 2004).

Table 2.4: MRS Findings for *myo*-Inositol and Phosphomonoesters in Major Depressed Patients.

Study	Technique	N[a]	Findings	Notes
Kato et al. 1992	1.5T ^{31}P-MRS DRESS	12	- ↑ frontal lobe PME in depressed UPD patients compared to euthymic BPD patients. - ↔ frontal lobe PME and PDE between depressed UPD patients and euthymic UPD patients and HC.	Various Medications
Moore et al. 1997	1.5T ^{31}P-MRS ISIS	35	- ↔ basal ganglia PME and PDE between depressed UPD patients and HC.	Drug-Free
Frey et al. 1998	1.5T ^{1}H-MRS STEAM	22	- ↓ right frontal lobe m-Ino in depressed UPD patients compared to HC. - ↔ left frontal lobe m-Ino between depressed UPD patients and HC.	Various Medications
Volz et al. 1998	1.5T ^{31}P-MRS ISIS	14	- ↑ frontal lobe PME in depressed UPD patients compared to HC. - Percentage of frontal lobe PME in depressed UPD patients was negatively correlated with degree of depression.	In-patients Various Medications
Auer et al. 2000	1.5T ^{1}H-MRS PRESS	19	- ↔ anterior cingulate cortex m-Ino between depressed UPD patients and HC. - ↔ parietal lobe white matter m-Ino between depressed UPD patients and HC.	Various Medications
Kumar et al. 2002	1.5T ^{1}H-MRS PRESS	20	- ↑ left DLPFC white matter m-Ino in depressed UPD patients compared to HC. - ↔ anterior cingulate grey matter m-Ino between depressed UPD patients and HC.	Elderly Drug-Free
Wyckoff et al. 2003	1.5T ^{1}H-MRS PRESS	8	- ↑ left DLPFC white matter m-Ino correlated with decreased magnetization transfer ratios in depressed UP patients but not in HC. - No such associations were found in anterior cingulate grey matter.	Elderly Drug-Free
Gruber et al. 2003	3T ^{1}H-MRS STEAM	17	- ↓ prefrontal cortex white matter m-Ino in depressed UPD patients compared to HC.	Drug-Free
Rosenberg et al. 2004	1.5T ^{1}H-MRS PRESS	14	- ↔ anterior cingulate cortex m-Ino between UPD patients and OCD patients and HC.	Drug-Naïve
Binesh et al. 2004	1.5T ^{1}H-MRS L-COSY	15	- ↔ prefrontal cortex white matter m-Ino between depressed UPD patients and HC.	Elderly Drug-Free
Elderkin-Thompson et al. 2004	1.5T ^{1}H-MRS L-COSY	14	- ↑ left DLPFC m-Ino correlated with increased cognitive function in HC but not in depressed UPD patients. - Change in left DLPFC m-Ino did not relate with changes in learning, recall, recognition, executive function, hypothesis generation, or processing speed.	Elderly Drug-Free
Coupland et al. 2005	3T ^{1}H-MRS STEAM	13	- ↓ dorsomedial prefrontal cortex m-Ino in UPD patients compared to HC.	Drug-Free

[a] The sample size represents the number of patients (not controls) in each study.

In medicated UPD patients, no differences in left frontal lobe *myo*-inositol concentrations were found when compared to HC (Frey et al. 1998); however, among UPD patients on antidepressant treatment, reduced *myo*-inositol levels were reported for the right frontal lobe (Frey et al. 1998). In a separate study, no differences in *myo*-inositol concentrations were reported for the anterior cingulate gyrus and parietal white matter between UPD patients and HC (Auer et al. 2000). Studies examining changes in PME concentrations in the frontal lobe of medicated UPD patients have also been mixed, with two studies showing no changes (Kato et al. 1992; Moore et al. 1997) while one study reported an increase in PME concentrations (Volz et al. 1998) between patients and HC.

2.2 MRS Findings in Healthy Volunteers

Several studies have assessed the effects of mood stabilizing medications in healthy volunteers (HV), and these findings are summarized in Table 2.5. In two studies, HV were administered lithium for 1 week, with no significant effect of lithium on frontal lobe *myo*-inositol concentrations observed (Silverstone et al. 1996, 1999). In addition, this group found no change in frontal lobe PME concentrations pre- and post-lithium (Silverstone et al. 1996, 1999), although an increase has been reported by another group (Yildiz et al. 2001). Moreover, in a recent study by Brambilla and colleagues (2004), they reported no difference in DLPFC *myo*-inositol concentrations following 4 weeks of lithium treatment.

In the same study, Silverstone and colleagues (1999) found that in lithium-treated HV given dextro-amphetamine [a putative human model for mania (Jacobs and Silverstone 1986)], frontal lobe PME, but not *myo*-inositol, concentrations increased. This finding may be consistent with the findings (discussed previously) of increased frontal lobe PME concentrations in manic (Kato et al. 1991, 1993) and hypomanic (Kato et al. 1994b) BPD patients.

Table 2.5: MRS Findings for *myo*-Inositol and Phosphomonoesters in Healthy Volunteers.

Study	Technique	N[a]	Findings	Notes
Silverstone et al. 1996	1.5T ^{1}H-MRS STEAM ^{31}P-MRS ISIS	12	- ↔temporal lobe m-Ino and PME in HV following lithium treatment.	Post-Lithium
Silverstone et al. 1999	3T ^{1}H-MRS PRESS ^{31}P-MRS ISIS	10	- ↔temporal lobe m-Ino and PME in HV following lithium treatment. - ↔temporal lobe m-Ino in HV following acute dextro-amphetamine. - ↑temporal lobe PME in lithium-treated HV following acute dextro-amphetamine. - ↔temporal lobe PME in placebo-treated HV following acute dextro-amphetamine.	Post-Lithium Acute dextro-amphetamine
Yildiz et al. 2001	1.5T ^{31}P-MRS Spin Echo	8	- ↑frontal lobe PME in lithium-treated HV.	Post-Lithium
Brambilla et al. 2004	1.5T ^{1}H-MRS STEAM	12	- ↔left and right DLPFC m-Ino in HV following lithium treatment.	Post-Lithium

[a] The sample size represents the number of volunteers in each study.

2.3 Summary of the MRS Findings

In considering all published studies to date, the evidence from studies of depressed BPD patients suggests that in both medicated and unmedicated patients there may be a decrease in *myo*-inositol concentrations in the frontal region (Frey et al. 1998; Moore et al. 1999), with no change occurring in the cingulate (Moore et al. 2000). There may be a similar decrease in depressed UPD patients (Gruber et al. 2003; Frey et al. 1998). In lithium-treated depressed BPD patients there are more consistent data suggesting increased PME concentrations in the frontal region (Kato et al. 1992, 1994b, 1995). In manic patients the findings to date suggest an increase in *myo*-inositol concentrations in untreated patients (Davanzo et al. 2001, 2003) and a possible decrease, or normalized level, in treated patients (Davanzo et al. 2001). PME concentrations also seem to be increased in most lithium-treated manic and hypomanic patients (Kato et al. 1991, 1993, 1994b). In contrast, taking the results in euthymic patients together, there is no evidence that lithium (or valproate) lowers *myo*-inositol concentrations and increases PME concentrations. However, since there may be no baseline changes, it is certainly conceivable that with the uncompetitive nature of lithium's inhibitory action on PI-cycle activity, lithium would have a more pronounced effect on this system when it is over-activated (evidenced by increases in *myo*-inositol concentration), which does not appear to be the case in bipolar euthymia. More support for this comes from HV studies where lithium administration had no effect on *myo*-inositol or PME concentrations at baseline in most (Brambilla et al. 2004; Silverstone et al 1996, 1999), but not all, studies (Yildiz et al. 2001). This finding appears to be independent of treatment length, with similar findings reported in both acute (Silverstone et al. 1996, 1999) and chronic (Brambilla et al. 2004) treatment studies. However, an increase in PME, but not *myo*-inositol, concentrations was seen following dextro-amphetamine administration (Silverstone et al. 1999). This is interesting given that dextro-amphetamine administration has been suggested as a model for mania (Jacobs and Silverstone 1986). Furthermore, a postmortem study also reported reduced *myo*-inositol in the frontal cortex of a mixed group of unmedicated BPD patients, when compared to HC (Shimon et al. 1997). It is still unclear whether there are differences in *myo*-inositol concentrations between the BPD subtypes and whether or not BPD and UPD patients have differences in PI-cycle functioning, this thesis aims to address the latter.

2.4 Thesis Objectives

As described in this chapter, *myo*-inositol and the PI-cycle have been implicated in the pathophysiology of mood disorders and in the mechanism of action of lithium. Previous work from this laboratory has shown that *myo*-inositol concentrations do not appear to be altered in euthymic BPD patients, nor in HV treated with lithium. Other groups have reported alterations in *myo*-inositol concentrations among manic, hypomanic and depressed BPD and UPD patients, but with less consistency. None of these studies have utilized methodology specifically designed to assess *myo*-inositol *in vivo*. Nor have these studies directly assessed the BPD subtypes or compared age- and sex-matched BPD and UPD depressed patients directly. Finally, it remains unknown if lithium's effect on *myo*-inositol metabolism is common among other mood stabilizing and anti-depressant medications. Given this dearth of information, this thesis aimed to further characterize the involvement of *myo*-inositol and the PI-cycle in mood disorders and their treatment. The series of experiments reported herein are unique and represent a thorough investigation of the importance of *myo*-inositol in mood regulation, examining its role in both the treatment and symptoms of mood disorders, from bench to bedside.

Based on these observations, the objectives of the present body of work were as follows:

(1) To determine whether treatment with lithium alters rat brain *myo*-inositol concentrations in a region-specific manner and whether treatment length is important in this process.

(2) To determine whether treatment with medication effective in BPD and UPD depression (i.e. mood stabilizers or antidepressants) will alter *myo*-inositol concentrations in rat brain.

(3) To assess, as a putative model for mania, the effects of acute dextro-amphetamine on brain *myo*-inositol concentration in HV and in rats.

(4) To assess *myo*-inositol concentrations in the dorsomedial prefrontal cortex of depressed BPD-II patients relative to age- and sex-matched HC.

(5) To assess *myo*-inositol concentrations in the dorsomedial prefrontal cortex of depressed UPD patients relative to age- and sex-matched HC.

(6) To contrast dorsomedial prefrontal cortex *myo*-inositol concentrations between depressed BPD-II patients with those in age- and sex-matched depressed UPD patients.

The subsequent chapters of this thesis follow the order of the objectives outlined above, moving literally from bench to bedside. The preclinical work is reported first, followed by the acute study of the effects of dextro-amphetamine in both humans and animals. Finally, the results from the study of BPD and UPD depression are reported. Both the preclinical and clinical studies employ magnetic resonance spectroscopy as the same methodological approach.

2.5 Bibliography

Allison JH, Blisner ME, Holland WH, Hipps PP, Sherman WR. Increased brain myo-inositol 1-phosphate in lithium-treated rats. Biochem Biophys Res Comm 1976;71:664-670.

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nat New Biol 1971;233:267-268.

Alvarez JC, Gluck N, Arnulf I, Quintin P, Leboyer M, Pecquery R, Launay JM, Perez-Diaz F, Spreux-Varoquaux O. Decreased platelet serotonin transporter sites and increased platelet inositol triphosphate levels in patients with unipolar depression: Effects of clomipramine and fluoxetine. Clin Pharmacol Ther 1999;66:617-624.

Auer DP, Putz B, Kraft E, Lipinski B, Schill J, Holsboer F. Reduced glutamate in the anterior cingulate cortex in depression: An in vivo proton magnetic resonance spectroscopy study. Biol Psychiatry 2000;47:305-313.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Binesh N, Kumar A, Hwang S, Mintz J, Thomas MA. Neurochemistry of late-life major depression: A pilot two-dimensional MR spectroscopic study. J Magn Reson Imaging 2004;20:1039-1045.

Brambilla P, Stanley JA, Sassi RB, Nicoletti MA, Mallinger AG, Keshavan MS, Soares JC. ^{1}H MRS study of dorsolateral prefrontal cortex in healthy individuals before and after lithium administration. Neuropsychopharmacol 2004;29:1918-1924.

Bruhn H, Stoppe G, Staedt J, Merboldt KD, Hanicke W, Frahm J. Quantitative proton MRS in vivo shows cerebral myo-inositol and cholines to be unchanged in manic-depressive patients treated with lithium. Proceedings of the Society of Magnetic Resonance in Medicine, August 14-20, 1993, New York. 1543.

Cecil KM, DelBello MP, Morey R, Strakowski SM. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord 2002;4:357-365.

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children and familial bipolar disorder. Biol Psychiatry 2003;53:1059-1065.

Coupland NJ, Ogilvie CJ, Hegadoren KM, Seres P, Hanstock CC, Allen PS. Decreased prefrontal myo-inositol in major depressive disorder. Biol Psychiatry 2005;57:1526-1534.

Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Brain metabolic alterations in medication-free patients with bipolar disorder. Arch Gen Psychiatry 2004;61:450-458.

Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M, McCracken J. Decreased anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. Neuropsychopharmacol 2001;24:359-369.

Davanzo P, Yue K, Thomas MA, Belin T, Mintz J, Venkatraman TN, Santoro E, Barnett S, McCracken J. Proton magnetic resonance spectroscopy of bipolar disorder versus intermittent explosive disorder in children and adolescents. Am J Psychiatry 2003;160:1442-1452.

Deicken RF, Fein G, Weiner MW. Abnormal frontal lobe phosphorus metabolism in bipolar disorder. Am J Psychiatry 1995a;152:915-918.

Deicken RF, Weiner MW, Fein G. Decreased temporal lobe phosphomonoesters in bipolar disorder. J Affect Disord 1995b;33:195-199.

Dwivedi Y, Janicak PG, Pandey GN. Elevated [^{3}H]inositol 1,4,5-trisphosphate binding sites and expressed inositol 1,4,5-trisphosphate receptor protein level in platelets of depressed patients. Psychopharmacol 1998;138:47-54.

Elderkin-Thompson V, Thomas MA, Binesh N, Mintz J, Haroon E, Dunkin JJ, Kumar A. Brain metabolites and cognitive function among older depressed and healthy individuals using 2D MR spectroscopy. Neuropsychopharmacol 2004;29:2251-2257.

Frey R, Metzler D, Fischer P, Heiden A, Scharfetter J, Moser E, Kasper S. Myo-inositol in depressive and healthy subjects determined by frontal ^{1}H-magnetic resonance spectroscopy at 1.5 tesla. J Psychiatr Res 1998;32:411-420.

Friedman SD, Dager SR, Parow A, Hirashima F, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Lithium and valproic acid treatment effects on brain chemistry in bipolar disorder. Biol Psychiatry 2004;56:340-348.

Grüber S, Frey R, Mlynarik V, Stadlbauer A, Heiden A, Kasper S, Kemp GJ, Moser E. Quantification of metabolic differences in the frontal brain of depressive patients and controls obtained by ^{1}H-MRS at 3 Tesla. Invest Radiol 2003;38:403-408.

Hamakawa H, Murashita J, Yamada N, Inubushi T, Kato N, Kato T. Reduced intracellular pH in the basal ganglia and whole brain measured by ^{31}P-MRS in bipolar disorder. Psychiatry Clin Neurosci 2004;58:82-88.

Jacobs D, Silverstone T. Dextroamphetamine-induced arousal in human subjects as a model for mania. Psychol Med 1986;16:323-329.

Kato T, Hamakawa H, Shioiri T, Murashita J, Takahashi Y, Takahashi S, Inubushi T. Choline-containing compounds detected by proton magnetic resonance spectroscopy in the basal ganglia in bipolar disorder. J Psychiatry Neurosci 1996;21:248-254.

Kato T, Ishiwata M, Mori K, Washizuka S, Tajima O, Akiyama T, Kato N. Mechanisms of altered Ca^{2+} signaling in transformed lymphoblastoid cells from patients with bipolar disorder. Int J Neuropsychopharmacol 2003;6:379-389.

Kato T, Kunugi H, Nanko S, Kato N. Association of bipolar disorder with the 5178 polymorphism in mitochondrial DNA. Am J Med Genet 2000;96:182-186.

Kato T, Murashita J, Kamiya A, Shioiri T, Kato N, Inubushi T. Decreased brain intracellular pH measured by ^{31}P-MRS in bipolar disorder: A confirmation in drug-free patients and correlation with white matter hyperintensity. Eur Arch Psychiatry Clin Neurosci 1998;248:301-306.

Kato T, Shioiri T, Murashita J, Hamakawa H, Inubushi T, Takahashi S. Phosphorus-31 magnetic resonance spectroscopy and ventricular enlargement in bipolar disorder. Psychiatry Res 1994a;55:41-50.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded 31P-MRS. Psychol Med 1995;25:557-566.

Kato T, Shioiri T, Takahashi S, Inubushi T. Measurement of brain phosphoinositide metabolism in bipolar patients using in vivo ^{31}P-MRS. J Affect Disord 1991;22:185-190.

Kato T, Takahashi S, Shioiri T, Inubushi T. Alterations in brain phosphorus metabolism in bipolar detected by in vivo ^{31}P and ^{7}Li magnetic resonance spectroscopy. J Affect Disord 1993;27:53-60.

Kato T, Takahashi S, Shioiri T, Inubushi T. Brain phosphorus metabolism in depressive disorders detected by phosphorus-31 magnetic resonance spectroscopy. J Affect Disord 1992;26:223-230.

Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. J Affect Disord 1994b;31:125-133.

Kim H, McGrath BM, Silverstone PH. A review of the possible relevance of inositol and the phosphatidylinositol second messenger system (PI-cycle) to psychiatric disorders – focus on magnetic resonance spectroscopy (MRS) studies. Hum Psychopharmacol Clin Exp 2005;20:309-326.

Kumar A, Thomas A, Lavretsky H, Yue K, Huda A, Curran J, Venkatraman T, Estanol L, Mintz J, Mega M, Toga A. Frontal white matter biochemical abnormalities in late-life major depression detected with proton magnetic resonance spectroscopy. Am J Psychiatry 2002;159:630-636.

Mikuni M, Kusumi I, Kagaya A, Kuroda Y, Mori H, Takahashi K. Increased 5-HT-2 receptor function as measured by serotonin-stimulated phosphoinositide hydrolysis in platelets of depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 1991;15:49-61.

Moore GJ, Bebchuk JM, Parrish JK, Faulk MW, Arfken CL, Strahl-Bevacqua J, Manji HK. Temporal dissociation between lithium-induced changes in frontal lobe myo-inositol and clinical response in manic-depressive illness. Am J Psychiatry 1999;156:1902-1908.

Moore CM, Breeze JL, Gruber SA, Babb SM, Frederick B, Villafuerte RA, Stoll AL, Hennen J, Yurgelun-Todd DA, Cohen BM, Renshaw PF. Choline, myo-inositol and mood in bipolar disorder: A proton magnetic resonance spectroscopy imaging study of the anterior cingulate cortex. Bipolar Disord 2000;2:207-216.

Moore CM, Christensen JD, Lafer B, Fava M, Renshaw PF. Lower levels of nucleoside triphosphate in the basal ganglia of depressed subjects: A phosphorus-31 magnetic resonance spectroscopy study. Am J Psychiatry 1997;154:116-118.

Murashita J, Kato T, Shioiri T, Inubushi T, Kato N. Altered brain energy metabolism in lithium-resistant bipolar disorder detected by photic stimulated ^{31}P-MR spectroscopy. Psychol Med 2000;30:107-115.

Pandey GN, Ren X, Pandey SC, Dwivedi Y, Sharma R, Janicak PG. Hyperactive phosphoinositide signaling pathway in platelets of depressed patients: Effect of desipramine treatment. Psychiatry Res 2001;105:23-32.

Rosenberg DR, Mirza Y, Russell A, Tang J, Smith JM, Banerjee SP, Bhandari R, Rose M, Ivey J, Boyd C, Moore GJ. Reduced anterior cingulated glutamatergic concentrations in childhood OCD and major depression versus healthy controls. J Am Acad Child Adolesc Psychiatry 2004;43:1146-1153.

Sharma R, Venkatasubramanian PN, Barany M, Davis JM. Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizoph Res 1992;8:43-49.

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE. Reduced frontal cortex inositol levels in postmortem brain of suicide victims and patients with bipolar disorder. Am J Psychiatry 1997;154:1148-1150.

Silverstone PH, Hanstock CC, Fabian J, Staab R, Allen PS. Chronic lithium does not alter human myo-inositol or phosphomonoester concentrations as measured by ^{1}H and ^{31}P MRS. Biol Psychiatry 1996;40:235-246.

Silverstone PH, McGrath BM, Kim H. Bipolar disorder and myo-inositol: A review of the magnetic resonance spectroscopy findings. Bipolar Disord 2005;7:1-10.

Silverstone PH, Rotzinger S, Pukhovsky A, Hanstock CC. Effects of lithium and amphetamine on inositol metabolism in the human brain as measured by ^{1}H and ^{31}P MRS. Biol Psychiatry 1999;46:1634-1641.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Human Psychopharmacol 2002;17:321-327.

Volz HP, Rzanny R, Riehemann S, May S, Hegewald H, Preussler B, Hubner G, Kaiser WA, Sauer H. ^{31}P magnetic resonance spectroscopy in the frontal lobe of major depressed patients. Eur Arch Psychiatry Clin Neurosci 1998;248:289-295.

Winsberg ME, Sachs N, Tate DL, Adalsteinsson E, Spielman D, Ketter TA. Decreased dorsolateral prefrontal N-acetylaspartate in bipolar disorder. Biol Psychiatry 2000;47:475-481.

Wyckoff N, Kumar A, Gupta RC, Alger J, Hwang S, Thomas MA. Magnetization transfer imaging and magnetic resonance spectroscopy of normal-appearing white matter in late-life major depression. J Magn Reson Imaging 2003;18:537-543.

Yildiz A, Demopulos CM, Moore CM, Renshaw PF, Sachs GS. Effect of lithium on phosphoinositide metabolism in human brain: A proton decoupled (31)P magnetic resonance spectroscopy study. Biol Psychiatry 2001;50:3-7.

Chapter 3. Effects of Lithium Treatment Length on Regional Rat Brain *myo*-Inositol Concentrations.

(A version of this chapter has been published in *NeuroReport* – see McGrath et al. 2006)

3.1 Introduction

Bipolar disorder is a severe and chronic mental illness, afflicting about 1% of the population. It is characterized by episodes of depression and mania. The most widely used long-term mood stabilizer for BPD patients is the monovalent cation, lithium (Fieve 1999). Lithium is effective in both the acute treatment of mania and the long-term prophylaxis of manic and depressive episodes (Post et al. 2000). This is important since the depressive symptoms are responsible for most of the morbidity and mortality in BPD patients (Baldassano et al. 2003).

Despite the recognition of several additional medications to treat bipolar disorder, the mechanism(s) of action of this diverse group of drugs remains uncertain. Understanding the mechanism(s) of action may provide insight into the aetiological basis of BPD (O'Donnell et al. 2000). The mechanism through which lithium exerts its therapeutic effect is unknown. One of the most widely studied hypotheses regarding lithium's mechanism of action is the inositol depletion hypothesis, which was based on the finding that lithium uncompetitively inhibits turnover of the PI-cycle, leading to a putative decrease in brain *myo*-inositol concentrations (Allison and Stewart 1971; Berridge and Irvine 1989; Berridge et al. 1982).

In neurons, the PI-cycle is activated following ligand binding with G_q-protein coupled receptors, including adrenergic (α_{1A} and α_{1B}), dopaminergic (D_1), serotonergic (5-HT_{1C} and 5-HT_2), and cholinergic (M_1 and M_3) receptor subtypes among others (Fisher et al. 1992) (Figure 1.1).

With agonist binding, the PI-cycle generates two intracellular second messengers, IP_3 and DAG. Optimal functioning is dependent on the successive recycling of *myo*-inositol. Many studies have shown effects of lithium upon the PI-cycle (Belmaker et al. 1998), and we and others have shown that both lithium and valproate may possibly have common effects on the PI-cycle in both the whole brain of animals (O'Donnell et al. 2000; Williams et al. 2002) and humans (Silverstone et al. 2002). However, because lithium is a centrally acting drug, its penetration and distribution in different regions of the brain have clinical and pathophysiological relevance (Ramaprasad 2004).

In the present study, we used ultra high-field ^{1}H-MRS NMR spectroscopy to examine the effects of lithium treatment length (1, 2 and 4 weeks) on changes in regional (prefrontal cortex, temporal cortex, occipital cortex and hippocampus) brain *myo*-inositol concentrations. To the authors' knowledge, this is the first report of the effects of lithium treatment length on regional *myo*-inositol concentrations. This is important, as clinical investigations in patients with BPD have rarely examined whole brain concentrations, focusing rather on changes in *myo*-inositol concentrations in regions implicated in the manifestation of mood. Moreover, this is the first study to assess rat brain metabolite concentrations using 18.8T ^{1}H-MRS.

3.2 Materials and Methods

3.2.1 Animals

Thirty-six adult male Sprague-Dawley rats (Biosciences Animal Service, University of Alberta), weighing 200–250 grams, were housed in pairs in standard Plexiglas laboratory cages. The rats were provided with food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*, and were maintained at 20°C, under a 12-h light/dark cycle (lights on 07:00–19:00 h), in a humidity-controlled environment. Treatment was started 1 week after the rats arrived, giving them an opportunity to acclimatize to their new environment. This study was reviewed and approved by the local Animal Policy and Welfare Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

3.2.2 Treatment

Each rat received a twice daily (b.i.d) intraperitoneal (IP) injection of either 1mmol/kg (2mmol/kg/day) lithium chloride (Fisher Scientific, Fair Lawn, NJ, USA) (n=18) in a 2ml/kg volume or vehicle (2ml/kg of saline) (n=18) at 07:30 h and 16:00 h. This dose of lithium chloride has been previously shown to produce therapeutic serum levels (Ghoshdastidar et al. 1989) and to inhibit *myo*-inositol production in a similar study of rat whole brain (O'Donnell et al. 2000) by our research group. The rats were separated into three groups based on treatment length: 1 week (n=12), 2 weeks (n=12) and 4 weeks (n=12). After treatment, rats were decapitated and the brains were rapidly removed and immediately immersed in ice-cold 2-methylbutane (Fisher Scientific, Fairlawn, NJ, USA). The brains were maintained at -80°C until brain dissection, tissue extraction and preparation for NMR analysis.

3.2.3 Brain Dissection and Preparation

Whole brains were dissected into pre-frontal, temporal and occipital cortex, as well as hippocampus according to stereotaxic demarcation (Pellegrino et al. 1979). Samples were prepared using a modified version (O'Donnell et al. 2000) of the extraction method described by Bligh and Dyer (1959). More specifically, brain regions were homogenized in 4 volumes of methanol/chloroform (2:1, v/v; Fisher Scientific, Fairlawn, NJ, USA). This was followed by the subsequent additions of one part of chloroform with homogenization, and one part of water with homogenization. A standardized amount of homogenate was transferred to an Eppendorf tube and centrifuged at 1000 rpm for 15 min in a bench top centrifuge (ThermoIEC, Needham Heights, MA, USA). Following centrifugation, a standardized amount of the water/methanol layer was transferred to a 12x75 mm Simport culture tube and maintained at -20°C overnight. The next day, samples were taken to dryness using vacuum centrifugation (Thermo Electron Corporation, Milford, MA, USA) and then returned to -20°C until NMR analysis.

At the time of NMR analysis, dried samples were reconstituted in 0.6 ml of dH_2O and 0.06 ml of D_2O containing 5 mM DSS as an internal reference standard (Markley et al. 1998), 100 mM imidazole and 0.2 % NaN_3 (Chenomx Inc., Edmonton, AB, CA), at a pH of approximately 7.

3.2.4 High-Field ^1H NMR Spectroscopy

NMR Spectroscopy was conducted at 37°C and 18.8T on a Varian Inova-800 spectrometer (Oxford Magnetics Inc., Oxford, England/Varian Inc., Palo Alto, USA) equipped with a 5 mm triple axis gradient HCN (^1H,^{13}C,^{15}N) probe. One-dimensional single 90° pulse ^1H spectra were collected with a water pre-saturation period of 2 seconds (γB_1 of ~ 150 Hz), sweep widths of 12000 Hz, and acquisition times of 2 seconds. All directly and indirectly detected data sets were zero filled to twice the number of acquired points. The 1D-^1H spectra were apodized using a 0.5 Hz line broadening or a cosine weighting function, respectively.

3.2.5 Identification and Quantification of Brain Metabolites

Identification and quantification of metabolites from brain extracts were done using Chenomx Profiler software (Chenomx Inc., Edmonton, AB, CA) on ^1H NMR spectra of brain extracts. Briefly, Profiler is linked to a database of metabolite molecules whose unique NMR spectral signatures are encoded at various spectrometer frequencies including 18.8 T (or 800 MHz). Comparison of the rat brain extract NMR spectra to the Chenomx spectral signature database within Profiler results in a list of compounds together with their respective concentrations. Metabolites were quantified by the addition of a known amount of the internal standard DSS (see above) to the brain extract samples, which also serves as a chemical shift reference (set to 0 ppm). All compounds in the database, including those discussed in this work, have been verified against known concentrations of reference NMR spectra of the pure compounds, enabling accurate metabolite identification and concentration quantification. Normalized metabolite concentrations were compared using the Independent samples t-test, with significance evaluated at the $\alpha=0.05$ level, using SPSS© (version 11.0.4 for OS X 10.4.2).

3.3 Results

3.3.1 Regional Differences in myo-Inositol Concentrations

Figure 3.1 depicts a typical ^{1}H spectrum of rat brain extract acquired at 18.8T, with the DSS peak referenced to 0 ppm. At 18.8T, *myo*-inositol gives multiple signals at 3.27, 3.52, 3.62, and 4.06 ppm. The spectral regions that contain contributions from *myo*-inositol (m-Ino) are expanded. Regional rat brain *myo*-inositol concentrations for 1, 2 and 4 weeks of lithium or vehicle administration are reported in table 3.1.

Figure 3.1: Typical ^{1}H MRS Spectrum from Rat Brain Extract highlighting the Resonances of *myo*-Inositol at 18.8 T.

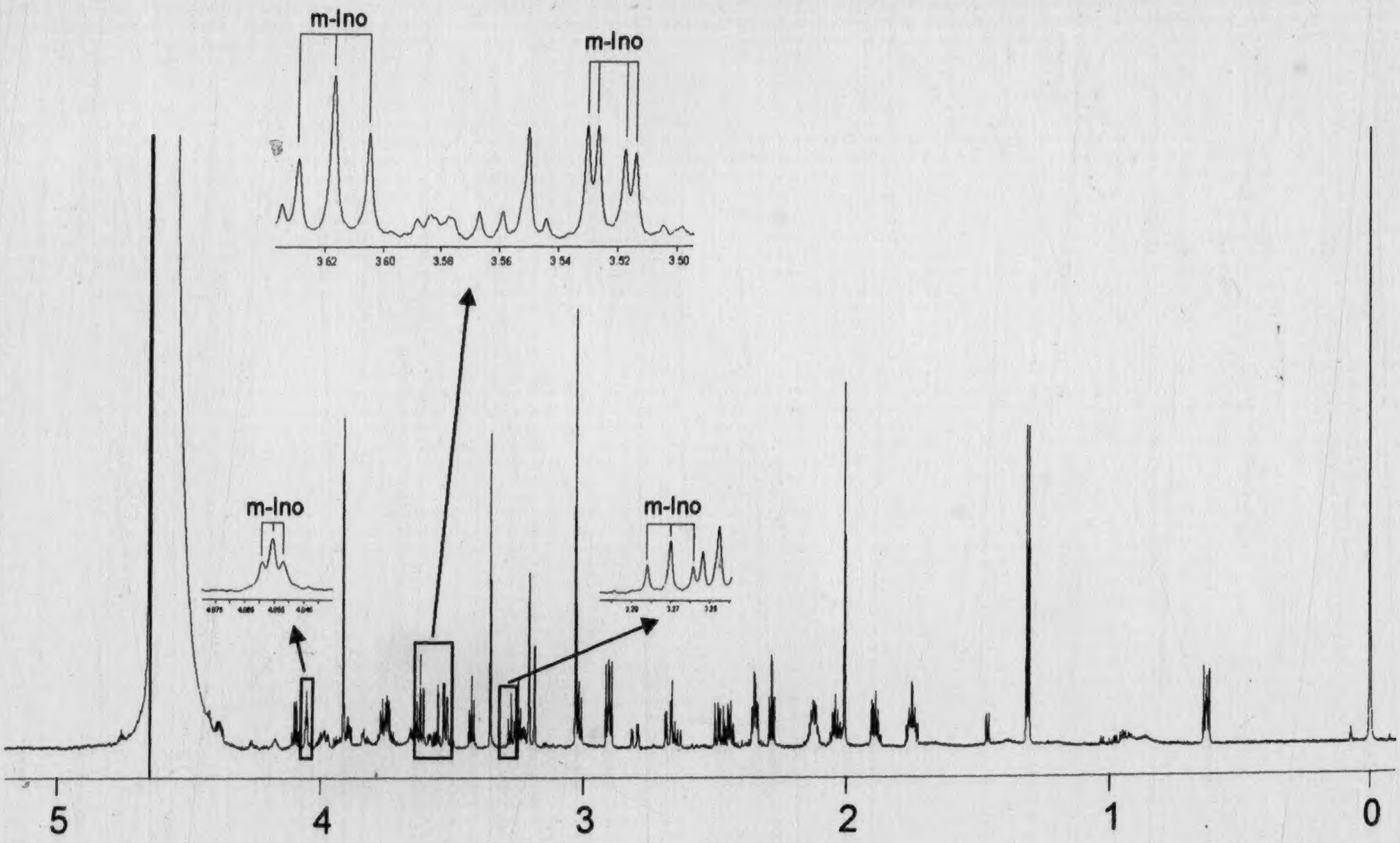


Table 3.1: *myo*-Inositol concentrations (μmol/gram wet weight) across Four Brain Regions, for Lithium- and Vehicle-Treated Rats.

Treatment Length	Treatment	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
1 week	Lithium	1.13 ± 0.19	4.12 ± 0.13	3.43 ± 0.22	3.31 ± 0.15
	Vehicle	1.17 ± 0.14	4.22 ± 0.09	3.62 ± 0.67	3.92 ± 0.30
2 weeks	Lithium	0.49 ± 0.04**	2.54 ± 0.06***	2.40 ± 0.16*	2.61 ± 0.23**
	Vehicle	0.94 ± 0.13	4.07 ± 0.14	3.22 ± 0.23	3.72 ± 0.10
4 weeks	Lithium	0.51 ± 0.04*	2.60 ± 0.15***	2.37 ± 0.25*	2.66 ± 0.12*
	Vehicle	0.92 ± 0.12	4.14 ± 0.12	3.14 ±0.13	3.72 ± 0.33

Lithium v. Vehicle: * $p<0.05$, ** $p<0.005$, *** $p<0.001$

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Metabolite concentrations are reported in micromoles (μmol) per gram of wet weight, normalized to the DSS standard concentration. Lithium did not alter *myo*-inositol concentrations across the four brain regions at 1 week of treatment when compared to vehicle-treated rats, although a non-significant reduction across all regions was evident (Table 3.1). However, there was a significant reduction in *myo*-inositol concentrations in lithium-treated rats at 2 and 4 weeks, across all four brain regions assessed, when compared to vehicle-treated rats (Table 3.1). The greatest reduction occurred at 2 weeks, with the *myo*-inositol concentration of the pre-frontal cortex in the lithium-treated rats showing the greatest overall reduction (44%), followed by hippocampus (38%), occipital cortex (30%) and temporal cortex (25%) (Figure 3.2 – 3.5). Concentration differences between treated and control rats at 4 weeks were relatively similar to those at 2 weeks (Table 3.1 and Figure 3.2 – 3.5).

Figure 3.2: Difference between Lithium- and Vehicle-Treated Rats in Pre-Frontal Cortex *myo*-Inositol concentrations (μmol/g wet weight).
Lithium v. Vehicle: * p<0.05, ** p<0.005, *** p<0.001.

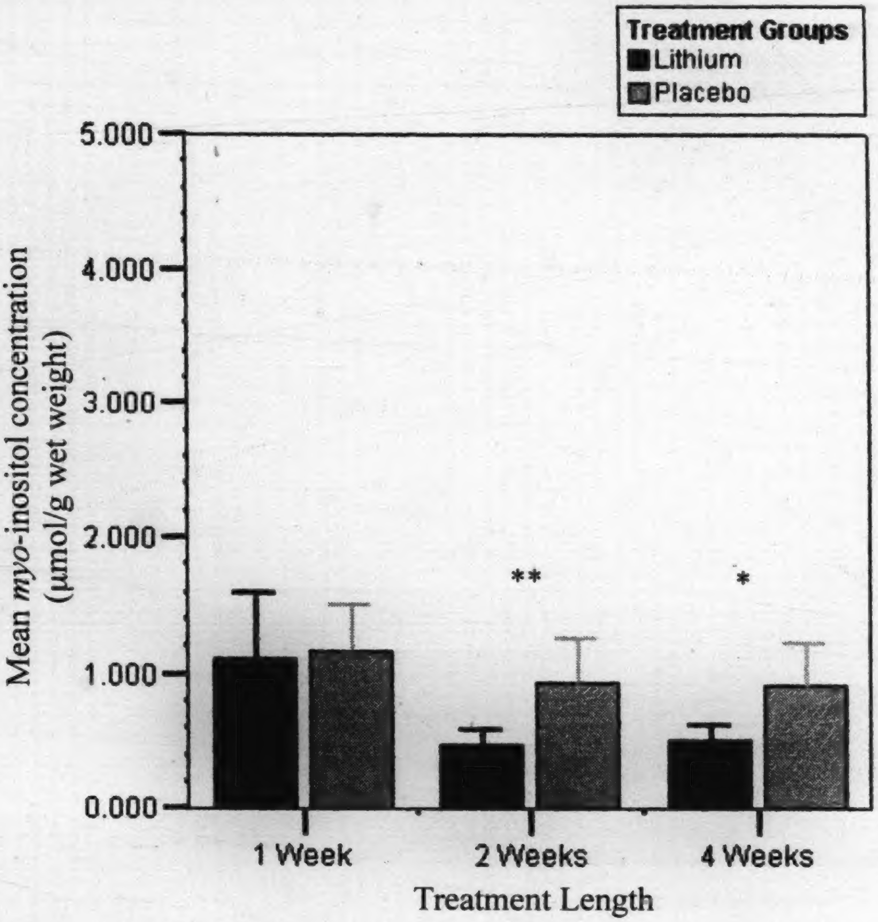


Figure 3.3: Difference between Lithium- and Vehicle-Treated Rats in Hippocampus *myo*-Inositol concentrations (µmol/g wet weight).

Lithium v. Vehicle: * $p<0.001$.

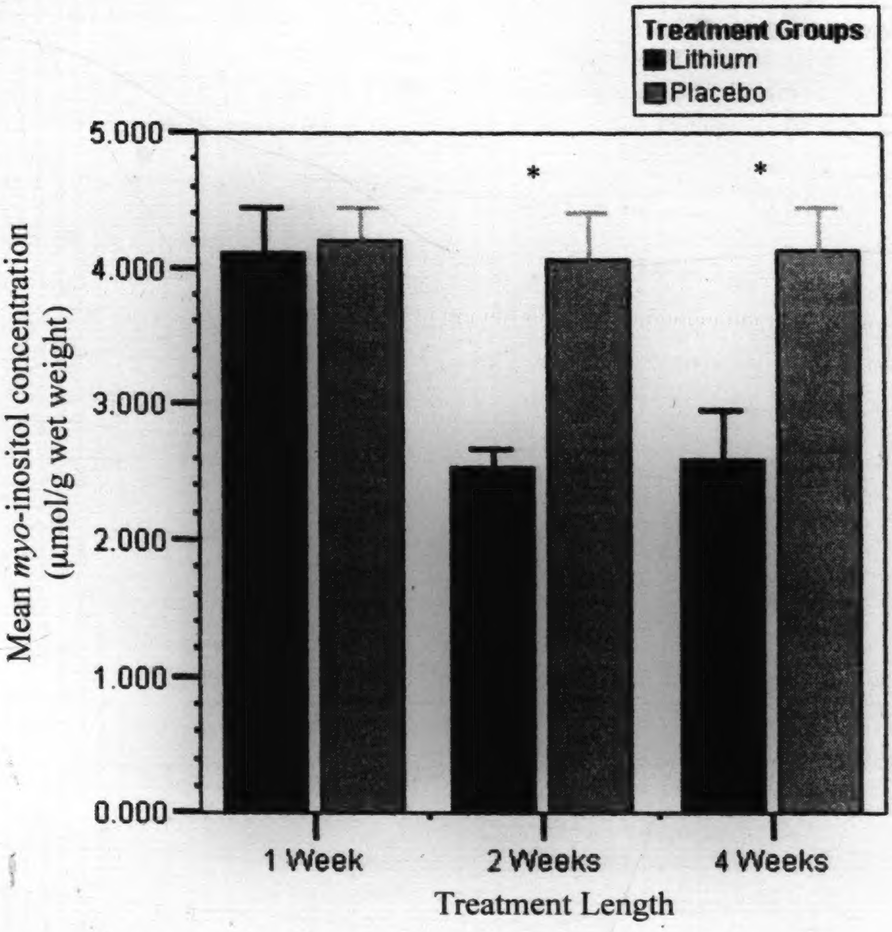


Figure 3.4: Difference between Lithium- and Vehicle-Treated Rats in Temporal Cortex *myo*-Inositol concentrations (µmol/g wet weight).

Lithium v. Vehicle: * $p<0.05$.

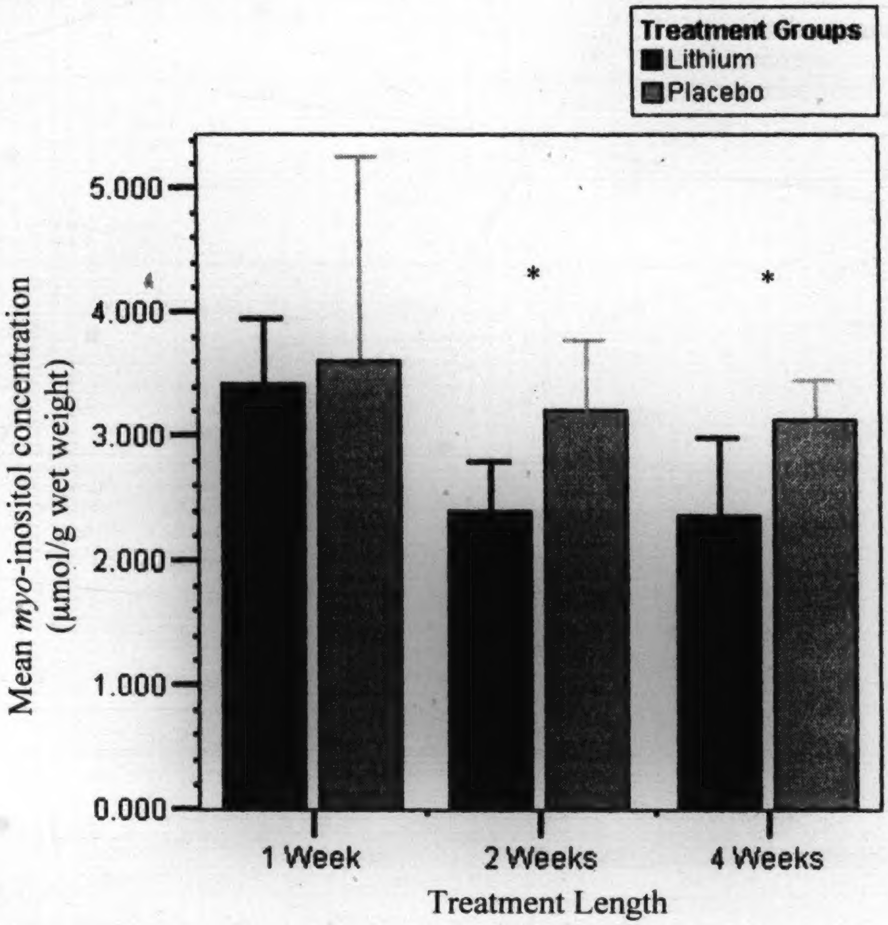
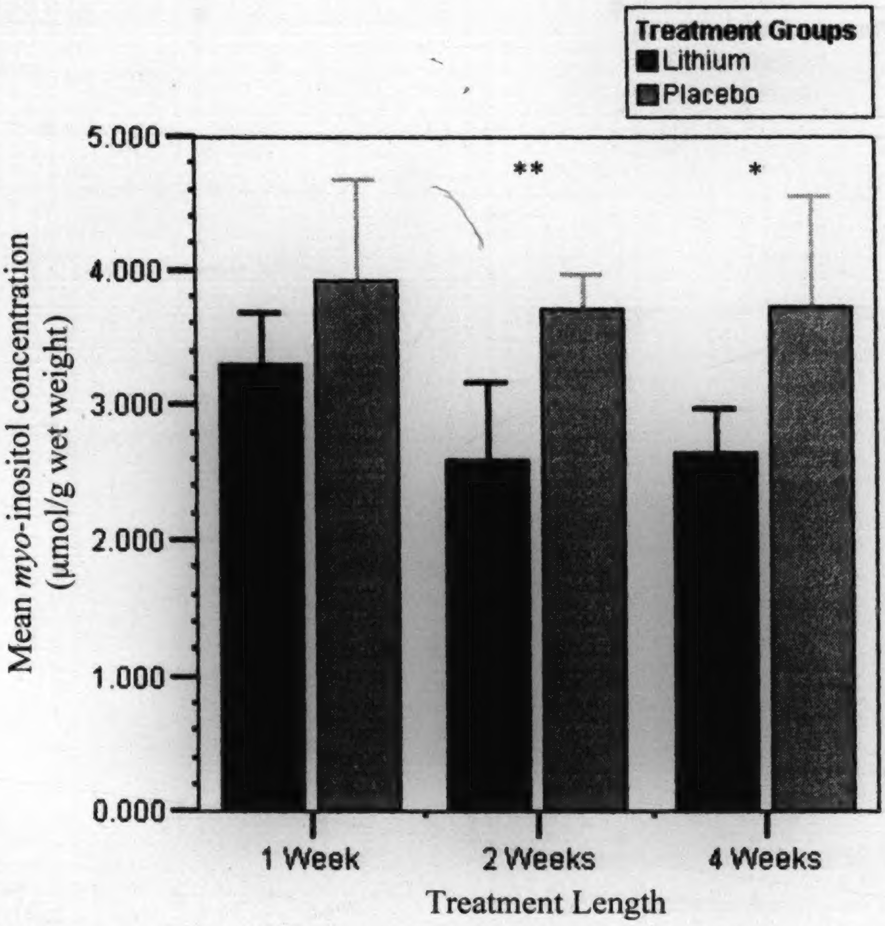


Figure 3.5: Difference between Lithium- and Vehicle-Treated Rats in Occipital Cortex *myo*-Inositol concentrations (µmol/g wet weight).

Lithium v. Vehicle: * $p<0.05$, ** $p<0.005$.



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3.4 Discussion

The inositol depletion hypothesis was originally proposed to explain lithium's clinical effectiveness following evidence of IMPase inhibition by clinically relevant concentrations of lithium (Allison and Stewart 1971; Berridge and Irvine 1989; Berridge et al. 1982). Our research group has previously reported that chronic (3 week) lithium treatment decreased the concentration of *myo*-inositol in whole rat brain (O'Donnell et al. 2000). Along with the use of a much more powerful magnet, the present body of work expands this earlier work in two ways: this is the first study to assess changes in regional *myo*-inositol concentrations as a function of treatment length; in addition, this is the first assessment of potential changes in multiple brain regions in this context, regions with known involvement in mood regulation.

The finding that lithium reduces *myo*-inositol after 2 and 4 weeks, but not after 1 week, is in line with the duration of lithium treatment required for symptomatic improvement in BPD (Revicki et al. 2005). Thus, the changes observed may be of clinical relevance. Treating rats at a dose that is known to produce therapeutic plasma levels similar to those occurring in patients (Ghoshdastidar et al. 1989; Hopkins and Gelenberg 2000) suggests that only those physiological changes that are induced by therapeutic treatment would be observed. This is particularly important with lithium due to its narrow therapeutic range (Dodds 2000). The lack of an effect at 1 week may be due, in part, to the slower accumulation of lithium in brain tissue relative to plasma (Lam and Christensen 1992), although some recent studies do not support this (Pearce et al. 2004; Plenge et al. 1994). The establishment of a steady-state may explain the plateau of the lithium effect at 2 and 4 weeks, as has been previously reported (Ghoshdastidar et al. 1989). There have also been reports of an increase in IMPase 1 activity following chronic (4 week) administration of a lithium-rich diet (Parthasarathy et al. 2003). This reported increase in IMPase 1 may mean that longer treatment (> 4 weeks) may not result in further reductions in *myo*-inositol concentration. It is also possible that *myo*-inositol levels may begin to increase with prolonged lithium treatment due to a compensatory production of IMPase 1. It has been suggested that this may be an adaptive response to lithium at the genomic level (Parthasarathy et al. 2003).

In relation to the distribution of *myo*-inositol changes induced by lithium in brain, the regions were chosen based on reports from the clinical literature showing alterations in *myo*-inositol concentrations in BPD patients. This was done to characterize more fully lithium's effects, and its relevance, if any, to BPD. Lithium reduced *myo*-inositol concentrations in each brain region assessed. This observation contrasts with previous reports in the clinical literature, where BPD patients have not shown consistent alterations in *myo*-inositol concentrations in the occipital lobe (reviewed in Silverstone et al. 2005). However, in a recent *in vivo* MRS study of lithium-7 (^{7}Li) distribution in rat brain at 7T, Ramaprasad (2004) reported that at therapeutic plasma levels, brain lithium levels are uniform. Lithium's effects on regional brain PI-cycle functioning may also be strain-dependent in rats, with Savolainen and associates (1990) reporting greater reductions in IP_1 (precursor to *myo*-inositol) across several brain regions in Sprague Dawley rats when compared to Han/Wistar rats. This suggests that the sensitivity of inositol phosphate phosphatases to inhibition by lithium may vary between strains (Savolainen et al. 1990). It is also possible that similar differences exist between species, and this may account for the regional differences in lithium's effects on *myo*-inositol concentration between clinical and preclinical reports. Consistent with our present findings, some researchers have reported that changes in inositol-1-phosphate concentrations occur across several brain regions (Savolainen et al. 1990; Hirvonen 1991).

In summary, while treatment with lithium for 1 week had no statistically significant effects on myo-inositol concentrations, treatment for 2 weeks and 4 weeks significantly reduced *myo*-inositol concentrations in rat brain when measured by ultra high-field ^{1}H-MRS. In addition, although previous studies (reviewed in chapter 2) suggest brain region-specific alterations in *myo*-inositol concentrations among BPD patients, our findings suggest that lithium-induced reduction of *myo*-inositol is more global and occurs in multiple regions within the rat brain. This finding, if confirmed, would not be supportive of theories of the mechanism of action of lithium that require region-specific effects. It remains unclear whether other mood-stabilizing agents have similar effects on *myo*-inositol concentrations, although some evidence exists for sodium valproate (O'Donnell et al. 2000). The following chapter will attempt to answer the question of whether this effect on *myo*-inositol is unique to lithium or if other mood stabilizers and antidepressants also influence the PI-cycle through reductions in *myo*-inositol concentrations.

3.5 Bibliography

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nature New Biol 1971;233:267-268.

Baldassano CF, Datto SM, Littman L, Lipari MA. What drugs are best for bipolar depression? Ann Clin Psychiatry 2003;15:225-232.

Belmaker RH, Agam G, van Calker D, Richards MH, Kofman O. Behavioral reversal of lithium effects by four inositol isomers correlates perfectly with biochemical effects on PI cycle: Depletion by chronic lithium of brain inositol is specific to hypothalamus, and inositol levels may be abnormal in postmortem brain from bipolar patients. Neuropsychopharmacol 1998;19:220-232.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Berridge MJ, Irvine RF. Inositol phosphates and cell signaling. Nature 1989;341:197-205.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-917.

Dodds G. Lithium therapy. Scott Med J 2000;45:171-173.

Fieve RR. Lithium therapy at the millennium: A revolutionary drug used for 50 years faces competing options and possible demise. Bipolar Disord 1999;1:67-70.

Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in the nervous system: An update. J Neurochem 1992;58:18-38.

Ghoshdastidar D, Dutta RN, Poddar MK. In vivo distribution of lithium in plasma and brain. Ind J Exp Biol 1989;27:950-954.

Hirvonen MR. Cerebral lithium, inositol and inositol monophosphates. Pharmacol Toxicol 1991;69:22-27.

Hopkins HS, Gelenberg AJ. Serum lithium levels and the outcome of maintenance therapy of bipolar disorder. Bipolar Disord 2000;2:174-179.

Lam HR, Christensen S. Regional and subcellular localization of Li+ and other cations in the rat brain following long-term lithium administration. J Neurochem 1992;59:1372-1380.

Markley JL, Bax A, Arata Y, Hilbers CW, Kaptein R, Sykes BD, Wright PE, Wuthrich K. Recommendations for the presentation of NMR structures of proteins and nucleic acids. IUPAC-IUBMB-IUPAB Inter-Union Task Group on the Standardization of Data Bases of Protein and Nucleic Acid Structures Determined by NMR Spectroscopy. J Biomolecul NMR 1998;12:1-23.

McGrath BM, Greenshaw AJ, McKay R, Slupsky CM, Silverstone PH. Lithium alters regional rat brain myo-inositol at 2 and 4 weeks: An ex vivo MRS study at 18.8 Tesla. NeuroReport 2006.

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH. Chronic lithium and sodium Valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 2000;880:84-91.

Parthasarathy LK, Seelan RS, Wilson MA, Vadnal RE, Parthasarathy RN. Regional changes in rat brain inositol monophosphatase 1 (IMPase 1) activity with chronic lithium treatment. Prog Neuro-Psychopharmacol Biol Psychiatry 2003;27:55-60.

Pearce JM, Lyon M, Komoroski RA. Localized 7Li MR Spectroscopy: In vivo brain and serum concentrations in the rat. Mag Reson Med 2004;52:1087-1092.

Pellegrino LJ, Pellegrino AS, Cushman AJ. A Stereotaxic Atlas of the Rat Brain, 2nd Edition. Plenum Press: New York, NY. 1979.

Plenge P, Stensgaard A, Jensen HV, Thomsen C, Mellerup ET, Henriksen O. 24-hour lithium concentration in human brain studied by Li-7 magnetic resonance spectroscopy. Biol Psychiatry 1994;36:511-516.

Post RM, Weiss SRB, Clark M, Chuang D-M, Hough C, Li H. Lithium, carbamazepine, and valproate in affective illness. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Revicki DA, Hirschfeld RM, Ahearn EP, Weisler RH, Palmer C, Keck PE Jr. Effectiveness and medical costs of divalproex versus lithium in the treatment of bipolar disorder: Results of a naturalistic clinical trail. J Affect Disord 2005;86:183-193.

Ramaprasad S. Lithium spectroscopy imaging of rat brain at therapeutic doses. Magn Reson Imag 2004;22:727-734.

Savolainen KM, Hirvonen M-R, Tarhanen J, Nelson SR, Samson FE, Pazdernik TL. Changes in cerebral inositol-1-phosphate concentrations in LiCl-treated rats: Regional and strain differences. Neurochem Res 1990;15:541-545.

Silverstone PH, McGrath BM, Kim H. Bipolar disorder and myo-inositol: A review of the magnetic resonance spectroscopy findings. Bipolar Disord 2005;7:1-10.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Hum Psychopharmacol Clin Exp 2002;17:321-327.

Williams RS, Cheng L, Mudge AW, Harwood AJ. A common mechanism of action for three mood-stabilizing drugs. Nature 2002;417:292-295.

Chapter 4. Effects of Two-Week Anticonvulsant and Antidepressant Treatment on Regional Rat Brain *myo*-Inositol Concentrations.

4.1 Introduction

Bipolar disorder is characterized by episodes of depression and mania. The most widely used long-term mood stabilizer for these patients is the monovalent cation, lithium (Fieve 1999). Lithium is effective in both the acute treatment of mania and the long-term prophylaxis of manic and depressive episodes (Post et al. 2000), but only in approximately 50% of bipolar patients (Muzina and Calabrese 2005; Davis et al. 1999; Kusumaker et al. 1997). This combined with lithium's narrow therapeutic window and side effect profile has helped drive the search for medications with similar therapeutic effects without these liabilities. Toward this end, other agents with mood-stabilizing properties – discovered serendipitously – have been introduced, with the most widely used being the anticonvulsants sodium valproate and carbamazepine (Muzina and Calabrese 2005; Calabrese 2000).

Recently, another anticonvulsant, lamotrigine, has been found to have efficacy in the treatment of the depressive phase of BPD (Gajwani et al. 2005; Calabrese et al. 2003; Calabrese et al. 1999). This is important since the depressive symptoms are responsible for most of the morbidity and mortality experienced by patients with BPD (Baldassano et al. 2003). However, there is little demonstrated efficacy for lamotrigine in the treatment of acute manic symptoms (Goldsmith et al. 2004). There is also recent evidence to suggest that antidepressants might be of comparable efficacy in the treatment of depression in both UPD and BPD (Gijsman et al. 2004), particularly in BPD-II depression (Amsterdam and Brunswick 2003).

Despite the recognition of several additional medications to treat BPD, the mechanism(s) of action of these diverse groups of drugs remains uncertain. One of the most widely studied hypotheses regarding possible neuropathophysiological changes are based on the findings that lithium uncompetitively inhibits turnover of the PI-cycle, leading to a putative decrease in brain *myo*-inositol concentrations (Allison and Stewart 1971; Berridge and Irvine 1989; Berridge et al. 1982). Briefly, in neurons, the PI-cycle is activated following ligand binding with G_q-protein coupled receptors, including adrenergic (α_{1A} and α_{1B}), dopaminergic (D_1), serotonergic (5-HT_{1C} and 5-HT_2), and cholinergic (M_1 and M_3) receptor subtypes among others (Fisher et al. 1992) (Figure 1.2). Many studies have shown effects of lithium upon the PI-cycle (Belmaker et al. 1998), and we and others have shown that both lithium and valproate may have common effects on the PI-cycle in animals (O'Donnell et al. 2000; Williams et al. 2002; McGrath et al. 2006) and humans (Silverstone et al. 2002). However, there is very little evidence available assessing the effects of lamotrigine and carbamazepine on the PI-cycle, and even less evidence evaluating the effects of antidepressant medications.

The present study was conducted in an effort to answer three questions: Firstly, do lamotrigine, carbamazepine, and sodium valproate have effects on regional brain *myo*-inositol concentrations consistent with those of lithium? Secondly, do antidepressants belonging to different classes alter regional brain *myo*-inositol concentrations, and in what way? Thirdly, do these agents alter *myo*-inositol concentrations in brain regions purported to be involved in mood regulation and that have been shown clinically to have altered *myo*-inositol (i.e. prefrontal cortex, temporal cortex and hippocampus), but not in other regions (i.e. occipital cortex)? The answers to these questions would both provide valuable insight into the possible mechanism of action of these medications in treatment of BPD and UPD and, by extension, into the pathophysiological underpinnings of these disorders.

4.2 Materials and Methods

4.2.1 Animals

This study was conducted as two separate sets of experiments, one with antidepressant treatment and the other with anticonvulsant treatment. Forty-eight (n=24 in antidepressant group, and n=24 in anticonvulsant group) adult male Sprague-Dawley rats (Biosciences Animal Service, University of Alberta), weighing 200–250 grams, were housed in pairs in standard Plexiglas laboratory cages. The rats were provided with food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*, and were maintained at 20°C, under a 12-h light/dark cycle (lights on 07:00–19:00 h), in a humidity-controlled environment. Treatment was started 1 week after the rats arrived, giving them an opportunity to acclimatize to their new environment. This study was reviewed and approved by the local Animal Policy and Welfare Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

4.2.2 Treatment

4.2.2.1 Anticonvulsant Group

Each rat received a single daily IP dose of, either 50mg/kg carbamazepine (Sigma Chemical Company, St. Louis, MO, USA) (n=6), 10mg/kg lamotrigine (GlaxoSmithKline, North Carolina, USA) (n=6), 300mg/kg sodium valproate (Sigma Chemical Company, St. Louis, MO, USA) (n=6), or vehicle (4ml/kg of propylene glycol) (n=6). Treatment length and drug doses were chosen based on other similar studies (Castel-Branco et al. 2003; Hasegawa et al. 2003; O'Donnell et al. 2000; Chen et al. 1999; Shaltiel et al. 2004; McGrath et al. 2006). All injections were administered in 4ml/kg volumes of propylene glycol (Fisher Scientific, Fairlawn, NJ, USA).

4.2.2.2 Antidepressant Group

Each rat received a single daily IP dose of, either 10mg/kg phenelzine (Sigma Chemical Company, St. Louis, MO, USA) (n=6), 5mg/kg fluoxetine (Sigma

Chemical Company, St. Louis, MO, USA) (n=6), 10mg/kg desipramine (Sigma Chemical Company, St. Louis, MO, USA) (n=6), or vehicle (2ml/kg of saline) (n=6). Treatment length and drug doses were chosen based on other similar studies (Dwivedi et al. 2002; Okada et al. 1988; Todd et al. 1995; McGrath et al. 2006). All injections were administered in 2ml/kg volumes of saline (Fisher Scientific, Fairlawn, NJ, USA).

4.2.3 Brain Dissection and Preparation

After 2 weeks, rats were decapitated and the brains were rapidly removed and immediately immersed in ice-cold 2-methylbutane (Fisher Scientific, Fairlawn, NJ, USA). The brains were maintained at -80°C until brain dissection, tissue extraction and preparation for NMR analysis. Whole brains were dissected into pre-frontal, temporal and occipital cortex, as well as hippocampus according to stereotaxic demarcation (Pellegrino et al. 1979). Samples were prepared using a modified version (O'Donnell et al. 2000) of the extraction method described by Bligh and Dyer (1959). More specifically, brain regions were homogenized in 4 volumes of methanol/chloroform (2:1, v/v; Fisher Scientific, Fairlawn, NJ, USA). This was followed by the subsequent additions of one part of chloroform with homogenization, and one part of water with homogenization. A standardized amount of homogenate was transferred to an Eppendorf tube and centrifuged at 1000 rpm for 15 min in a bench top centrifuge (ThermoIEC, Needham Heights, MA, USA). Following centrifugation, a standardized amount of the water/methanol layer was transferred to a 12x75 mm Simport culture tube and maintained at -20°C overnight. The next day, samples were taken to dryness using vacuum centrifugation (Thermo Electron Corporation, Milford, MA, USA) and then returned to -20°C until NMR analysis.

At the time of NMR analysis, dried samples were reconstituted in 0.6 ml of dH_2O and 0.06 ml of D_2O containing 5 mM DSS as an internal reference standard (Markley et al. 1998), 100 mM imidazole and 0.2 % NaN_3 (Chenomx Inc., Edmonton, AB, CA), at a pH of approximately 7.

4.2.4 High-Field ^1H NMR Spectroscopy

NMR Spectroscopy was conducted at 37°C and 18.8T on a Varian Inova-800 spectrometer (Oxford Magnetics Inc., Oxford, England/Varian Inc., Palo Alto, USA) equipped with a 5 mm triple axis gradient HCN probe. One-dimensional single 90° pulse ^{1}H spectra were collected with a water pre-saturation period of 2 seconds (γB_1 of ~ 150 Hz), sweep widths of 12000 Hz, and acquisition times of 2 seconds. All directly and indirectly detected data sets were zero filled to twice the number of acquired points. The 1D-^{1}H spectra were apodized using a 0.5 Hz line broadening or a cosine weighting function, respectively.

4.2.6 Identification and Quantification of Brain Metabolites

Identification and quantification of metabolites from brain extracts were done using Chenomx Profiler software (Chenomx Inc., Edmonton, AB, CA) on ^{1}H NMR spectra of brain extracts. Briefly, Profiler is linked to a database of metabolite molecules whose unique NMR spectral signatures are encoded at various spectrometer frequencies including 18.8 T (or 800 MHz). Comparison of the rat brain extract NMR spectra to the Chenomx spectral signature database within Profiler results in a list of compounds together with their respective concentrations. Metabolites were quantified by the addition of a known amount of the internal standard DSS (see above) to the brain extract samples, which also serves as a chemical shift reference (set to 0 ppm). All compounds in the database, including those discussed in this work, have been verified against known concentrations of reference NMR spectra of the pure compounds, enabling accurate metabolite identification and concentration quantification. Normalized metabolite concentrations were compared using the one-way analysis of variance (ANOVA), with significance evaluated at the α=0.05 level, using SPSS© (version 11.0.4 for OS X 10.4.2).

4.3 Results

4.3.1 Treatment Effects on Regional myo-Inositol Concentrations

Figure 3.1 depicts a typical ^1H spectrum of rat brain extract acquired at 18.8T, with the DSS peak referenced to 0 ppm. At 18.8T, *myo*-inositol gives multiplet signals at 3.27, 3.52, 3.62, and 4.06 ppm. The spectral regions that contain contributions from *myo*-inositol (m-Ino) are expanded. Regional rat brain *myo*-inositol concentrations for anticonvulsant treatment are reported in Table 4.1 and concentrations for antidepressant treatment are reported in Table 4.2.

Metabolite concentrations are reported in μmol per gram of wet weight, normalized to the DSS standard concentration. No significant differences between the *myo*-inositol concentrations in frontal, temporal and occipital cortex and hippocampus were observed for lamotrigine-, sodium valproate- and carbmazapine-treated groups when compared to vehicle [One-way analysis of variance (ANOVA), p>0.05]. Similarily, treatment with desipramine, phenelzine or fluoxetine did not significantly alter *myo*-inositol concentrations relative to vehicle (One-way ANOVA, p>0.05). This is interesting, in that these belong to distinctly different classes (tricyclic, monoamine oxidase inhibitor, and selective serotonin reuptake inhibitor, respectively).

Table 4.1: *myo*-Inositol Concentrations (μmol/gram wet weight) across Four Brain Regions, for Anticonvulsant- (Lamotrigine, Sodium Valproate and Carbamazepine) and Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
Treatment:				
Vehicle	1.07 ± 0.20	3.13 ± 0.66	1.81 ± 0.14	2.44 ± 0.67
Lamotrigine	1.06 ± 0.25	3.07 ± 0.70	2.07 ± 0.36	2.01 ± 0.26
Sodium Valp.	1.36 ± 0.27	3.78 ± 1.22	2.10 ± 0.32	1.99 ± 0.15
Carbamazepine	1.13 ± 0.10	2.18 ± 0.21	1.69 ± 0.19	1.81 ± 0.18

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Table 4.2: *myo*-Inositol Concentrations (μmol/gram wet weight) across Four Brain Regions, for Antidepressant- (Desipramine, Fluoxetine and Phenelzine) and Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
Treatment:				
Vehicle	0.74 ± 0.10	3.78 ± 0.58	3.55 ± 0.48	2.64 ± 0.37
Desipramine	1.44 ± 0.32	2.70 ± 0.33	3.44 ± 0.75	3.25 ± 0.48
Fluoxetine	1.26 ± 0.22	2.73 ± 0.20	3.12 ± 0.83	3.88 ± 0.36
Phenelzine	0.84 ± 0.18	3.51 ± 0.56	2.54 ± 0.45	2.10 ± 0.34

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

4.4 Discussion

The inositol depletion hypothesis was originally proposed to explain lithium's clinical effectiveness following evidence that it inhibits inositol monophosphatase (Allison and Stewart 1971; Berridge and Irvine 1989; Berridge et al. 1982). It has previously been shown that chronic lithium treatment decreases the concentration of *myo*-inositol in whole rat brain (O'Donnell et al. 2000) and more recently, in the hippocampus, pre-frontal, temporal and occipital cortices (McGrath et al. 2006). Thus, it was of interest to determine whether this effect on *myo*-inositol is unique to lithium, or whether it might be a shared mechanism of medications used in the treatment of BPD. There is some preclinical evidence to support a similar mechanism with sodium valproate (O'Donnell et al. 2000). Moreover, given that depression is the most often experienced symptom, and that there is evidence to suggest altered *myo*-inositol concentrations in both BPD and UPD depressed patients, it was of interest to determine whether antidepressant medications also affect *myo*-inositol levels, and whether this is dependent on antidepressant class.

The main finding from the present study was that there were no differences found when comparing anticonvulsant-treated (lamotrigine, sodium valproate or carbamazepine) or antidepressant-treated (desipramine, phenelzine or fluoxetine) rats with vehicle-treated rats on concentrations of *myo*-inositol, across the four brain regions assessed. This is the first study to assess the direct effects of these medications on *myo*-inositol concentrations using high-field MRS. While it is possible that lithium exerts its therapeutic effect by direct inhibition of *myo*-inositol production, the mechanism by which sodium valproate, carbamazepine and lamotrigine exert their effects is less certain.

With the exception of lithium, most of the research into the actions of mood stabilizers on *myo*-inositol metabolism has focused on sodium valproate, with early evidence suggesting that this agent may reduce *myo*-inositol in yeast (Vaden et al. 2001) and in the whole brain of rats (O'Donnell et al. 2000), similar to the effects of lithium. More recent work on sodium valproate indicates that it may in fact decrease PI-cycle activity, but not *myo*-inositol concentration, through a direct inhibition of the enzyme prolyl oligopeptidase (PO) (Cheng et al. 2005). Although the exact intracellular substrate for PO is unknown, there is evidence suggesting that PO may inhibit the PI-cycle (Williams et al. 1999; Schulz et al. 2002) and that bipolar patients may even have altered PO activity (Breen et al. 2004). Valproate may also inhibit the *de novo* production of *myo*-inositol by an indirect inhibition of the enzyme myo-inositol-1-phosphate synthase (Shaltiel et al. 2004); however, expression of this enzyme is restricted to the brain's vasculature (Wong et al. 1987), so the effects of its inhibition on neuronal concentration of *myo*-inositol are unclear. Incidentally, carbamazepine does not show this effect on PO (Cheng et al. 2005). A separate line of reasoning suggests that lithium may, in fact, work via its inhibition of glycogen synthase kinase-3 (GSK-3)-mediated signal transduction (O'Brien et al. 2004; Phiel and Klein 2001), with recent evidence for valproate as well (Werstuck et al. 2004; Kim et al. 2005). How GSK-3 is involved in bipolar pathophysiology is not clear, as most of the work has focused on studies of GSK-3 in non-mammalian species or in cell culture (Lenox and Wang 2003). Lithium's effect on GSK-3 may be independent and/or unrelated to lithium's effects on *myo*-inositol concentration. There is evidence that GSK-3 mediates microtubule assembly and the stabilization of synapses (Hong et al. 1997; Klein and Melton 1996) and that inhibitors of GSK-3 mimic the therapeutic action of mood stabilizers (Eldar-Finkelman 2002). There is also recent evidence that lamotrigine may be effective in bipolar treatment as a result of its inhibition of both rapidly-firing action potentials and excessive glutamate release – normalizing overexcited, but not basal, neuronal activities (Hahn et al. 2004). This is consistent with this author's own work, which did not show changes in basal glutamate concentration following lamotrigine treatment (see Appendix A). To this author's knowledge, this is the first study that directly assesses the effects of lamotrigine on PI-cycle metabolism via changes in *myo*-inositol.

Few studies have assessed *myo*-inositol metabolism in patients with unipolar depression, and even fewer have evaluated the effects of different classes of antidepressant medication on *myo*-inositol concentrations. Antidepressants have long been known to effect serotonergic and noradrenergic pathways in the brain. However, there is a growing body of evidence suggesting that these effects are not mediated by a single transmitter system. For instance, fluoxetine has recently been shown to increase the concentration of the neurosteroid, allopregnanolone, in mice at doses too low to inhibit 5-HT reuptake (Pinna et al. 2006). Moreover, some antidepressants, including desipramine, have been shown to down-regulate 5-HT_{2A} and β-adrenergic receptors (Syvalahti et al. 2006; Goodnough and Baker 1994; Pandey et al. 1985); however, fluoxetine does not (Goodnough and Baker 1994). As a result of these findings, investigations have moved beyond the level of the cell receptors, assessing the affects of antidepressants on signal transduction pathways (or second messenger systems). Most of the attention to date has focused on the adenylyl cyclase (cAMP) system and its downstream cellular and nuclear affects (D'Sa et al. 2005; Malberg and Blendy 2005). However, given the commonality between the receptors effected by antidepressant medications and those linked to the PI second messenger system, the PI-cycle and *myo*-inositol are valid candidates for involvement in the mechanism of antidepressant action. Indeed, there is evidence suggesting that some antidepressants decrease PI-cycle activity (Osborne 1988; Subhash and Jagadeesh 1997), and that the three antidepressants assessed in the present study down-regulate PI-phospholipase C (PI-PLC) activity and the expression of the PLCβ1 isozyme. From the current results, it appears that while desipramine, phenelzine and fluoxetine all alter PI-PLC, they do not affect *myo*-inositol concentrations. As a result, while they may affect the responsiveness of the PI-cycle, these antidepressants do not appear to alter the absolute concentration of *myo*-inositol available.

Considering the above discussion, and the findings from the present set of experiments, there are three possible explanations. First, it is possible that these agents affect the PI-cycle using a different mechanism than that of lithium, whereby they may change the tone or responsiveness of the PI second messenger system to agonist binding, which may not necessarily reduce basal *myo*-inositol concentration per se, but would have a similar effect to that of inositol depletion by reducing or altering its activity. This remains to be clarified however. Second, it is also possible that while each of these medications have been shown to affect the PI-cycle, these actions may be unrelated to the mechanism(s) involved in the treatment of mood disorders. Alternatively, and perhaps most likely, is that while many of these agents act on the PI-cycle, this action, in combination with other effects – from the level of the receptor to the nucleus – work synergistically to improve the symptoms experienced during periods of symptom exacerbation (Malberg and Blendy 2005) and that the effects on *myo*-inositol and the PI-cycle are not, in themselves, indicators of mood stabilizer or antidepressant action.

It is unclear to what extent the PI-cycle, and *myo*-inositol, are involved in the pathophysiology and treatment of mood disorders, and particularly unipolar depression. It appears that lithium has a non-specific effect, altering *myo*-inositol concentrations in many brain regions (McGrath et al. 2006), including in areas (i.e. occipital cortex) not typically associated with mood disorders or with *myo*-inositol changes in patients (reviewed in Silverstone et al. 2005). Similarly, from the present set of experiments it appears that commonly prescribed anticonvulsants and antidepressants do not change *myo*-inositol concentrations, but may affect the PI-cycle through other mechanisms. Again, from the literature, these effects do not appear to be region-specific, but rather are more global. This weakens the notion that the PI-cycle in general, and *myo*-inositol in particular, are aetiologically involved in the symptomatology of bipolar and unipolar mood disorders.

4.5 Bibliography

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nature New Biol 1971;233:267-268.

Amsterdam JD, Brunswick DJ. Antidepressant monotherapy for bipolar type II major depression. Bipolar Disord 2003;5:388-395.

Baldassano CF, Datto SM, Littman L, Lipari MA. What drugs are best for bipolar depression? Ann Clin Psychiatry 2003;15:225-232.

Belmaker RH, Agam G, van Calker D, Richards MH, Kofman O. Behavioral reversal of lithium effects by four inositol isomers correlates perfectly with biochemical effects on PI cycle: Depletion by chronic lithium of brain inositol is specific to hypothalamus, and inositol levels may be abnormal in postmortem brain from bipolar patients. Neuropsychopharmacol 1998;19:220-232.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Berridge MJ, Irvine RF. Inositol phosphates and cell signaling. Nature 1989;341:197-205.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physio 1959;37:911-917.

Breen G, Harwood AJ, Gregory K, Sinclair M, Collier D, St. Clair D, Williams RS. Two peptidase activities decrease in treated bipolar disorder not schizophrenic patients. Bipolar Disord 2004;6:156-161.

Calabrese JR. Efficacy of lamotrigine in bipolar disorder. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Calabrese JR, Bowden CL, Sachs G, Yatham LN, Behnke K, Mehtonen OP, Montgomery P, Ascher J, Paska W, Earl N, DeVeaugh-Geiss J, and the Lamictal 605 Study Group. A vehicle-controlled 18-month trail of lamotrigine and lithium maintenance treatment in recently depressed patients with bipolar I disorder. J Clin Psychiatry 2003;64:1013-1024.

Calabrese JR, Bowden CL, Sachs G, Ascher JA, Monaghan E, Rudd GD. A double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. J Clin Psychiatry 1999;60:79-88.

Castel-Branco M, Lebre V, Falcao A, Figueiredo I, Caramona M. Relationship between plasma and brain levels and the anticonvulsant effect of lamotrigine in rats. Eur J Pharmacol 2003;482:163-168.

Chen G, Huang LD, Jiang YM, Manji HK. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. J Neurochem 1999;72:879–882.

Cheng L, Lumb M, Polgar L, Mudge AW. How can the mood stabilizer VPA limit both mania and depression? Mol Cell Neurosci 2005;29:155-161.

Davis JM, Janicak PG, Hogan DM. Mood stabilizers in the prevention of recurrent affective disorders: A meta-analysis. Acta Psychiatrica Scandinavia 1999;100:406-417.

D'Sa C, Eisch AJ, Bolger GB, Duman RS. Differential expression and regulation of the cAMP-selective phosphodiesterase type 4A splice variants in rat brain by chronic antidepressant administration. Eur J Neurosci 2005;22:1463-1475.

Dwivedi Y, Agrawal AK, Rizavi HS, Pandey GN. Antidepressants reduce phosphoinositide-specific phospholipase C (PI-PLC) activity and the mRNA and protein expression of selective PLC β1 isozyme in rat brain. Neuropharmacol 2002;43:1269-1279.

Eldar-Finkelman H. Glycogen synthase kinase 3: An emerging therapeutic target. Trends Mol Med 2002;8:126-132.

Fieve RR. Lithium therapy at the millennium: A revolutionary drug used for 50 years faces competing options and possible demise. Bipolar Disord 1999;1:67-70.

Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in the nervous system: An update. J Neurochem 1992;58:18-38.

Gajwani P, Forsthoff A, Muzina D, Amann B, Gao K, Elhaj O, Calabrese JR, Grunze H. Antiepileptic drugs in mood-disordered patients. Epilepsia 2005;46(Suppl 4):38-44.

Gijsman HJ, Geddes JR, Rendell JM, Nolen WA, Goodwin GM. Antidepressants for bipolar depression: A systematic review of randomized, controlled trials. Am J Psychiatry 2004;161:1537-1547.

Goldsmith DR, Wagstaff AJ, Ibbotson T, Perry CM. Spotlight on lamotrigine in bipolar disorder. CNS Drugs 2004;18:63-67.

Goodnough DB, Baker GB. 5-Hydroxytryptamine 2 and β-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. J Neurochem 1994;62:2262-2268.

Hahn C-G, Gyulai L, Baldassano CF, Lenox RH. The current understanding of lamotrigine as a mood stabilizer. J Clin Psychiatry 2004;65:791-804.

Hasegawa H, Osada K, Misonoo A, Morinobu S, Yamamoto H, Miyamoto E, Asakura M. Chronic carbamazepine treatment increases myristoylated alanine-rich C kinase substrate phosphorylation in the rat cerebral cortex via down-regulation of calcineurin Aα. Brain Res 2003;994:19-26.

Hong M, Chen DC, Klein PS, Lee VM. Lithium reduces tau phosphorylation: Effects in living cells and in neurons at therapeutic concentrations. Biol Psychiatry 1999;45:995-1003.

Kim AJ, Shi Y, Austin RC, Werstuck GH. Valproate protects cells from ER stress-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3. J Cell Sci 2005;118:89-99.

Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci USA 1996;93:8455-8459.

Kusumaker V, Yatham LN, Haslam DR, Parikh SV, Matte R, Silverstone PH, Sharma V. Treatment of mania, mixed states, and rapid cycling. Can J Psychiatry 1997;42:79S-86S.

Lenox RH, Wang L. Molecular basis of lithium action: Integration of lithium-responsive signaling and gene expression networks. Mol Psychiatry 2003;8:135-144.

Malberg JE, Blendy JA. Antidepressant action: To the nucleus and beyond. Trends Pharmacol Sci 2005;26:631-638.

Markley JL, Bax A, Arata Y, Hilbers CW, Kaptein R, Sykes BD, Wright PE, Wuthrich K. Recommendations for the presentation of NMR structures of proteins and nucleic acids. IUPAC-IUBMB-IUPAB Inter-Union Task Group on the Standardization of Data Bases of Protein and Nucleic Acid Structures Determined by NMR Spectroscopy. J Biomolecul NMR 1998;12:1-23.

McGrath BM, Greenshaw AJ, McKay R, Slupsky CM, Silverstone PH. Lithium alters regional rat brain myo-inositol at 2 and 4 weeks: An ex vivo MRS study at 18.8 Tesla. NeuroReport 2006.

Muzina DJ, Calabrese JR. Maintenance therapies in bipolar disorder: Focus on randomized controlled trials. Aust New Zeal J Psychiatry 2005;39:652-661.

O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S, Klein PS. Glyogen synthase kinase-3beta haploinsufficiency mimics the behavioural and molecular effects of lithium. J Neurosci 2004;24:6791-6798.

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH. Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 2000;880:84-91.

Okada F, Tokumitou Y, Ui M. Possible involvement of pertussis toxin substrates (Gi, Go) in desipramine-induced refractoriness of adenylate cyclase in cerebral cortices of rats. J Neurochem 1988;51:194-199.

Osborne NN, Tobin AB, Ghazi H. Role of inositol trisphosphate as a second messenger in signal transduction processes: An essay. Neurochem Res 1988;13:177-191.

Pandey GN, Sudershan P, Davis JM. Beta adrenergic receptor function in depression and the effect of antidepressant drugs. Acta Pharmacol Toxicol 1985;56(Suppl 1):66-79.

Pellegrino LJ, Pellegrino AS, Cushman AJ. A Stereotaxic Atlas of the Rat Brain, 2nd Edition. Plenum Press: New York, NY. 1979.

Phiel CJ, Klein PS. Molecular targets of lithium action. Ann Rev Pharmacol Toxicol 2001;41:789-813.

Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. Psychopharmacol 2006;186:362-372.

Post RM, Weiss SRB, Clark M, Chuang DM, Hough C, Li H. Lithium, carbamazepine, and valproate in affective illness. In: Bipolar Medications: Mechanisms of Action. Manji H, Bowden CL, Belmaker RH (eds.). American Psychiatric Press Inc.: Washington, DC. 2000.

Schulz I, Gerhartz B, Neubauer A, Holloschi A, Heiser U, Hafner M, Demuth HU. Modulation of inositol 1,4,5-trisphosphate concentration by prolyl endopeptidase inhibition. Eur J Biochem 2002;269:5813-5820.

Shaltiel G, Shamir A, Shapiro J, Ding D, Dalton E, Bialer M, Harwood AJ, Belmaker RH, Greenberg ML, Agam G. Valproate decreases inositol biosynthesis. Biol Psychiatry 2004;56:868-874.

Silverstone PH, McGrath BM, Kim H. Bipolar disorder and myo-inositol: A review of the magnetic resonance spectroscopy findings. Bipolar Disord 2005;7:1-10.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Hum Psychopharmacol Clin Exp 2002;17:321-327.

Subhash MN, Jagadeesh S. Imipramine-induced changes in 5-HT2 receptor sites and inositoltrisphosphate levels in rat brain. Neurochem Res 1997;22:1095-1099.

Syvalahti E, Penttila J, Majasuo H, Palvimaki EP, Laakso A, Hietala J. Combined treatment with citalopram and buspirone: Effects on serotonin 5-HT2A and 5-HT2C receptors in the rat brain. Pharmacopsychiatry 2006;39:1-8.

Todd KG, McManus DJ, Baker GB. Chronic administration of the antidepressants phenelzine, desipramine, clomipramine, or maprotiline decrease binding of 5-hydroxytryptamine2A receptors without affecting benzodiazepine binding sites in rat brain. Cell Mol Neurobiol 1995;15:361-370.

Vaden DL, Ding D, Peterson B, Greenberg ML. Lithium and valproate decrease inositol mass and increase expression of the yeast INO1 and INO2 genes for inositol biosynthesis. J Biol Chem 2001;276:15466-15471.

Werstuck GH, Kim AJ, Brenstrum T, Ohnmacht SA, Panna E, Capretta A. Examining the correlations between GSK-3 inhibitory properties and anticonvulsant efficacy of valproate and valproate-related compounds. Bioorg Med Chem Lett 2004;14:5465-5467.

Williams RS, Cheng L, Mudge AW, Harwood AJ. A common mechanism of action for three mood-stabilizing drugs. Nature 2002;417:292-295.

Williams RS, Eames M, Ryves WJ, Viggars J, Harwood AJ. Loss of a prolyl oligopeptidase confers resistance to lithium by elevation of inositol (1,4,5) trisphosphate. EMBO J 1999;18:2734-2745.

Wong YH, Kalmbach SJ, Hartman BK, Sherman WR. Immunohistochemical staining and enzyme activity measurements show myo-inositol-1-phosphate synthase to be localized in the vasculature of brain. J Neurochem 1987;48:1434-1442.

Chapter 5. Modeling Mania: Effects of Acute dextro-Amphetamine Administration on *myo*-Inositol Concentrations in the Brains of Humans and Rats.

(A version of this chapter has been presented at the 61st annual meeting of the Society of Biological Psychiatry – see McGrath et al. 2006)

5.1 Introduction

Bipolar disorder affects at least 1% of the population, and it is characterized by manic and depressive episodes accompanied by severe disturbances in cognition, perception and behavior (McElroy and Keck 1996). While lithium has remained the mainstay for BPD treatment, other structurally dissimilar medications have been found to be effective in acute and prophylactic treatment, particularly sodium valproate (Geddes et al. 2004; Bowden 2003; Weisler et al. 2004; Herman 2004; Tohen et al. 2003). Nonetheless, the pathophysiological underpinnings that underlie these symptoms are not fully understood, although one of the most widely studied hypotheses regarding possible pathophysiological changes are based on the findings that lithium inhibits turnover of the PI-cycle in an uncompetitive manner (Allison and Stewart 1971; Berridge et al. 1982; Berridge and Irvine 1989).

In neurons, the PI-cycle has been found to be activated following ligand binding with G_q-protein coupled receptors, including adrenergic (α_{1A} and α_{1B}), dopaminergic (D_1), serotonergic (5-HT_{1C} and 5-HT_2), and cholinergic (M_1 and M_3) receptor subtypes among others (Fisher et al. 1992) (Figure 1.1). Many studies have shown effects of lithium upon the PI-cycle (Belmaker et al. 1998), and we and others have shown that both lithium and valproate may have common effects on the PI-cycle in both animals (O'Donnell et al. 2000; Williams et al. 2002) and humans (Silverstone et al. 2002a).

Despite these hypotheses, it can be hard to study mania, particularly when employing a methodology like MRS which often requires the patient to remain still for prolonged periods, and for this reason dextro-amphetamine has been used as a model for mania in several human studies as it causes similar subjective and physiological symptoms (Jacobs and Silverstone 1986; Angrist et al. 1987; Zacny et al. 1992; Silverstone et al. 1992; Brauer and de Wit 1996; Fabian and Silverstone 1997; Silverstone et al. 1998; Asghar et al. 2003). Given that the primary action of dextro-amphetamine is to increase extra-cellular concentrations of dopamine and noradrenaline (Hoebel et al. 1989; Seiden et al. 1993; Karler et al. 1994; Kuczenski and Segal 1997; Reid et al. 1997), it is likely to have indirect effects on the PI-cycle. This has been supported by studies in both animals (Barkai et al. 1981; Yu et al. 2003) and humans (Silverstone et al. 2002a). Furthermore, in human studies we have shown that pre-treatment with both lithium and valproate partially attenuates a range of dextro-amphetamine-induced changes in brain activation, cognition, and PI-cycle activity (Bell et al. 2005; Willson et al. 2005; Silverstone et al. 2002b). We surmised that this represented stimulation by dextro-amphetamine of the PI-cycle, and subsequent blockade of this effect by lithium and valproate.

Despite these positive findings, however, the effects of dextro-amphetamine on the PI-cycle are not certain. In one animal study of whole rat brain, O'Donnell and associates (2000) did not find any effects of dextro-amphetamine on the PI-cycle, although the author's hypothesized that this may have been due to underlying regional changes being masked by examining whole brain. Furthermore, in two earlier studies in HV the same group found no effects of dextro-amphetamine on the PI-cycle (Silverstone et al. 1996, 1999), although it was considered that in at least one case this may represent differences in magnetic resonance spectroscopy (MRS) techniques.

The present set of experiments was undertaken in an effort to address the issues raised above, and to determine if dextro-amphetamine induces effects on the PI-cycle, consistent with the inositol-depletion hypothesis of BPD. The techniques for detection of changes in *myo*-inositol concentrations in humans utilizing MRS had also improved, allowing us to examine these conflicting results in greater detail. The hypotheses for the present study were, therefore, that dextro-amphetamine would cause regional brain *myo*-inositol changes in rats, opposite to the whole brain effects reported for lithium and valproate. Secondly, we hypothesized that dextro-amphetamine administration would also lead to detectable frontal *myo*-inositol changes in HV, as measured by a novel MRS technique specifically designed to measure *myo*-inositol concentrations in human brain (Kim et al. 2005). The frontal brain region is of particular interest in the context of BPD symptomatology and *myo*-inositol metabolism, given that several studies have reported alterations in *myo*-inositol and its metabolic precursor in the frontal lobe of manic and hypomanic patients (Kato et al. 1991, 1993, 1994) as well as in HV given lithium (Yildiz et al. 2001). To this author's knowledge, this is the first clinical MRS study to utilize a pulse sequence specifically developed to detect *myo*-inositol. This is also the first study to report concurrent results from clinical and preclinical experiments assessing analogous physiologically processes using similar methodologies. If supported, these hypotheses would clearly define a mechanism linking putative changes in the PI-cycle to the subjective psychological changes seen with dextro-amphetamine administration, potentially further supporting the importance of the PI-cycle in mood disorders. This would also allow for direct contrast with alterations, if any, observed in the depressed phase of BPD.

5.2 Materials and Methods

5.2.1 Pre-Clinical Experiment

Twelve adult male Sprague-Dawley rats (Biosciences Animal Service, University of Alberta), weighing 350–450 grams, were housed in pairs in standard Plexiglas laboratory cages. The rats were provided with food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*, and were maintained at 20°C, under a 12-h light/dark cycle (lights on 07:00–19:00 h), in a humidity-controlled environment. Treatment was started 1 week after the rats arrived, giving them an opportunity to acclimatize to their new environment. This component of the study was reviewed and approved by the local Animal Policy and Welfare Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

5.2.1.1 Treatment

Each rat received a single, acute IP injection of either 5 mg/kg (based on free base weight) dextro-amphetamine (n=6) (Health and Welfare Canada, Ottawa, ON, CA) or saline (n=6). This dose was chosen based on other similar studies (Del Arco et al. 1998; Anderzhanova et al. 2001; Mcgeehan et al. 2004; Choe et al. 2002; Andiarzhanova et al. 2002; Anderzhanova et al. 2002). All injections were administered in 2 ml/kg volumes of saline. From previous work (Anderzhanova et al. 2002; Mora and Porras 1993), at 60 minutes post injection rats were decapitated. The brains were rapidly removed and immediately immersed in ice-cold 2-methylbutane (Fisher Scientific, Fairlawn, NJ, USA). The brains were maintained at -80°C until brain dissection, tissue extraction and preparation for NMR analysis.

5.2.1.2 Brain Dissection and Preparation

Whole brains were dissected into pre-frontal, temporal and occipital cortex, as well as hippocampus according to stereotaxic demarcation (Pellegrino et al. 1979). Samples were prepared using a modified version (O'Donnell et al. 2000) of the extraction method described by Bligh and Dyer (Bligh and Dyer 1959). More specifically, brain regions were homogenized in 4 volumes of methanol/chloroform (2:1, v/v; Fisher Scientific, Fairlawn, NJ, USA). This was followed by the subsequent additions of one part of chloroform with homogenization, and one part of water with homogenization. A standardized amount of homogenate was transferred to an Eppendorf tube and centrifuged at 1000 rpm for 15 min in a bench top centrifuge (ThermoIEC, Needham Heights, MA, USA). Following centrifugation, a standardized amount of the water/methanol layer was transferred to a 12x75 mm Simport culture tube and maintained at -20°C overnight. The next day, samples were taken to dryness using vacuum centrifugation (Thermo Electron Corporation, Milford, MA, USA) and then returned to -20°C until NMR analysis.

At the time of NMR analysis, dried samples were reconstituted in 0.6 ml of dH_2O and 0.06 ml of D_2O containing 5 mM DSS as an internal reference standard (Markley et al. 1998), 100 mM imidazole and 0.2 % NaN_3 (Chenomx Inc., Edmonton, AB, CA), at a pH of approximately 7.

5.2.1.3 High-Field ^1H NMR Spectroscopy

NMR Spectroscopy was conducted at 37°C and 18.8T on a Varian Inova-800 spectrometer (Oxford Magnetics Inc., Oxford, England/Varian Inc., Palo Alto, USA) equipped with a 5 mm triple axis gradient HCN probe. One-dimensional single 90° pulse ^1H spectra were collected with a water pre-saturation period of 2 seconds (γB_1 of ~ 150 Hz), sweep widths of 12000 Hz, and acquisition times of 2 seconds. All directly and indirectly detected data sets were zero filled to twice the number of acquired points. The 1D-^1H spectra were apodized using a 0.5 Hz line broadening or a cosine weighting function, respectively.

5.2.1.4 Identification and Quantification of Brain Extract Metabolites

Identification and quantification of metabolites from brain extracts were done using Chenomx Profiler software (Chenomx Inc., Edmonton, AB, CA) on ^1H NMR spectra of brain extracts. Briefly, Profiler is linked to a database of metabolite molecules whose unique NMR spectral signatures are encoded at various spectrometer frequencies including 18.8T (or 800 MHz). Comparison of the rat brain extract NMR spectra to the Chenomx spectral signature database within Profiler results in a list of compounds together with their respective concentrations. Metabolites were quantified by the addition of a known amount of the internal standard DSS (see above) to the brain extract samples, which also serves as a chemical shift reference (set to 0 ppm). All compounds in the database, including those discussed in this work, have been verified against known concentrations of reference NMR spectra of the pure compounds, enabling accurate metabolite identification and concentration quantification. Normalized metabolite concentrations were compared using the Independent samples t-test, with significance evaluated at the $\alpha=0.05$ level, using SPSS© (version 11.0.4 for OS X 10.4.2).

5.2.2 Clinical Experiment

5.2.2.1 Subjects

This component of the study was approved by the local human research ethics board of the University of Alberta Hospital, and all participants gave full informed consent. 12 HV (5 male and 7 female) were recruited through a newsletter advertisement from the university community. Volunteers underwent a brief medical history, and a detailed semi-structured clinical interview (structured clinical interview for DSM-IV Axis I disorders – SCID) to rule out past or present psychiatric illness. Volunteers were also assessed on the Hamilton depression rating scale (HAM-D), the Montgomery-Asberg depression rating scale (MADRS) and the Young mania rating scale (YMRS). Past and present drug and alcohol use was assessed and volunteers were excluded based on abuse of these substances. In addition, volunteers who had used recreational drugs in the past 6 months, or amphetamine in the past year were excluded from participating. A magnetic resonance safety screen was also performed to ensure safety in undergoing the scan procedure. At 12:00am on the day of the scan, volunteers were required to fast until both scans were complete.

5.2.2.2 Study Design

An open-label study design was used. All volunteers underwent a baseline MRS scan. Upon completion of the first scan, volunteers were administered a single oral 25 mg dose of dextro-amphetamine (GlaxoSmithKline, Montreal, PQ, CA). The second scan began 75 min after administration of dextro-amphetamine, which coincides with the peak subjective response to dextro-amphetamine, which occurs 60–120 min after administration (Asghar et al. 2003).

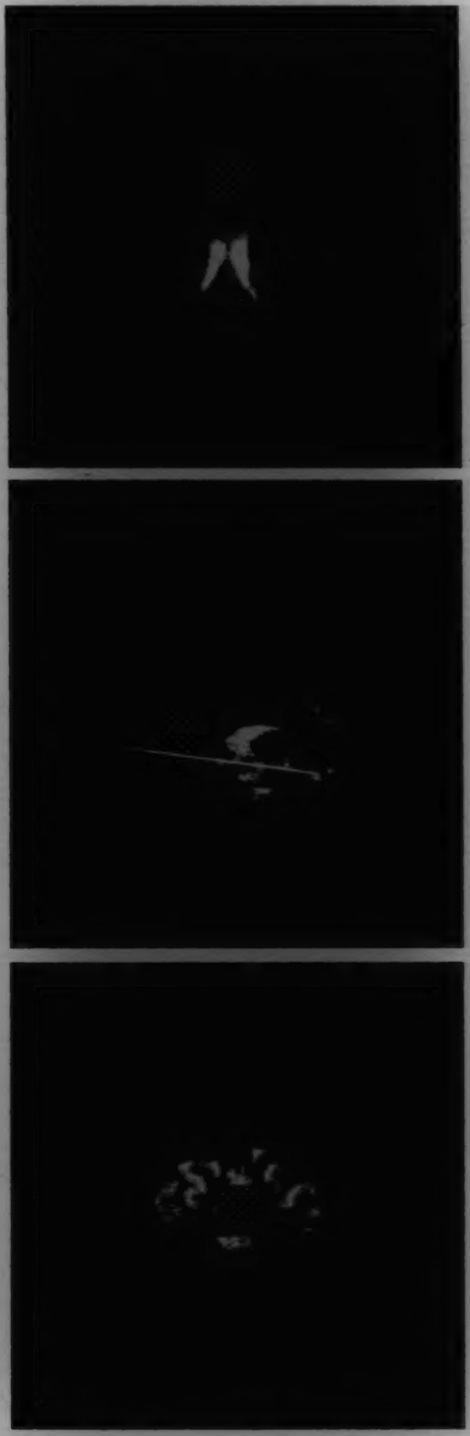
5.2.2.3 Physiologic and Subjective Response Measures

Resting heart rate and both systolic and diastolic blood pressure were measured at baseline and at 75 and 150 min after dextro-amphetamine administration. Subjective measurements of mood were also assessed at baseline and at 75 and 150 min after dextro-amphetamine using a 100 mm visual analogue scale (VAS) (Folstein and Luria 1973). The percentage change in VAS scores from baseline are reported.

5.2.2.4 Clinical ^1H MRS

Data were acquired with a 3.0T scanner (Magnex Scientific, Abingdon, United Kingdom/Surrey Medical Imaging Systems, Guilford, United Kingdom) with a 28-cm quadrature birdcage resonator for transmission and reception. Multi-slice gradient echo images (repetition time [TR] = 500 msec, echo time [TE] = 22 msec, slice thickness = 5 mm, 11 slices, resolution = 256 × 256) were used to co-register a 2.5 x 2.5 x 2.5-cm point-resolved spectroscopy (PRESS) voxel that encompassed dorsomedial prefrontal cortex (DMPFC). The lower border was orientated to a line connecting the anterior and posterior commissures. The voxel was centered on the midline, touching the tip of the genu of the corpus callosum (Figure 5.1). The PRESS-selected volume was used for both tissue segmentation and for acquiring water-suppressed metabolite spectra. Shimming was carried out first with FASTMAP (Gruetter 1993) to optimize the linear and non-linear shims over a 5-cm-diameter spherical volume, then with an automatic optimization of the linear *x, y, z* shims on the PRESS-selected voxel at TE1 = 36 msec, TE2 = 160 msec and TR = 3000 msec. Typical shimmed water line-widths were better than 6 Hz (0.05 ppm). The PRESS acquisitions were performed as the sum of 16 sub-spectra, each of 32 averages, allowing re-registration to the same frequency reference (the acetyl peak of NAA at 2.023 ppm) to eliminate the effects of frequency drift during the course of the acquisition.

Figure 5.1: Localization of a Single Voxel in the DMPFC.



5.2.2.4.1 Tissue Segmentation

Tissue segmentation into grey matter (GM), white matter (WM), and CSF was performed with a double inversion recovery sequence to acquire one-dimensional (1D) projections of each longitudinal relaxation time (T_1) compartment in the PRESS-selected volume (Hanstock and Allen 2000). Two hyperbolic secant inversion pulses (110-msec length, bandwidth = 150 Hz) were added to the PRESS pulse sequence, in which the pulses were 90° sinc-gauss and 180° optimized-sinc shapes. Before the 90° pulse, a 15-msec spoiler gradient was applied to dephase any transverse magnetization resulting from the inversion pulses. The PRESS parameters were TR = 9 sec, TE = 120 msec, two averages with 5-kHz sample frequency, digitized over 128 data points. Prior data were used to estimate the T_1 values for the three brain compartments (GM: 1070 ± 60 msec; WM: 720 ± 30 msec; CSF: 4440 ± 50 msec). With the expression derived by Redpath and Smith (1994), two pairs of T_{inv1} and T_{inv2} timings were computed that gave signal nulls of the CSF compartment with either that from either GM or WM. Twenty-one 1D projections were acquired, with ten projections for each set of double-null inversion timings, and one with just CSF nulled. An additional 10 1D projections were acquired with no inversion pulses and with a TE of 500 msec. This minimized the signal contamination from GM and WM (<0.2% residual signal after accounting for transverse relaxation time (T_2) losses), while maintaining significant signal from CSF (approximately 50% residual signal).

After phase correction of each projection, three-dimensional surface and contour maps were generated to confirm that the selected T_{inv1} and T_{inv2} timings were resulting in simultaneous nulls of CSF with either GM or WM. Subsequently, GM and WM projections were selected from the double inversion recovery 1D projection series. The T_{inv1} and T_{inv2} timings that were used to acquire these two series of projections for either GM or WM were then used to estimate a normalization factor that reflected attenuation due to T_1 and T_2 losses for each projection, thereby fully accounting for all the acquisition timings. Similarly, a normalization factor was estimated for the CSF projection. Segmentation resulted from first normalizing and then summing the signal across each of the three projections, such that the relative proportions of GM, WM, and CSF could be estimated. These proportions were then used to calculate a grey matter fraction [GM/(GM + WM)] for the voxel. All computations were performed within the MATLAB program environment (MathWorks, Natick, Massachusetts).

5.2.2.4.2 Identification and Quantification of Brain Metabolites

Internal water data used as the reference magnetic resonance (MR) signal standard for metabolite quantification were acquired using stimulated echo acquisition mode (STEAM) sequence at different TE values (TR=12 sec, TM=30 msec). Three STEAM acquisition series were performed, each as the sum of 2 averages for each TE value, for a total of 18 different TE values. Echo times in the first acquisition ranged from 20 msec – 100 msec, with a step size of 20 msec. Echo times in the second acquisition ranged from 150 msec – 500 msec, with a step size of 50 msec. Echo times in the third acquisition ranged from 700 msec – 1500 msec, with a step size of 200 msec.

Metabolite quantification was achieved using the signal from brain water, and by utilizing the following three series of data. These included: metabolite (M) peak area estimates, extracted from Linear Combination of Metabolite Basis Spectra (LCModel) program (Provencher 1993) output (M_{TE196}); segmentation information for GM, WM and CSF compartment sizes, used to estimate water (W) concentration in the selected brain voxel (W_{brain}), and; internal water data acquired at different TE values used as the reference MR signal standard (W_{TE0}).

Metabolite spectra were Fourier transformed and analyzed with LCModel. The basis spectra for LCModel analysis were simulated within the spectral bandwidth of 1.00–3.90 ppm with numeric methods (Hanstock et al. 2002). The metabolites included in the basis spectra were NAA, *N*-acetylaspartyl-glutamate, Cr, choline, glutamate, glutamine, GABA, *myo*-inositol, glycine, taurine, and aspartate. The in vivo metabolite data were accepted if the S/N ratio was 11 or more, and the standard deviation of the fit for the metabolite was $\leq 20\%$. In the present study, no subjects were excluded based on the aforementioned criteria.

For quantification, the water data were first imported into the processing software, and filtered, Fourier transformed, phase and baseline corrected. The water peak area from each spectrum in the TE series was determined and these area data were fitted to a multi-exponential using a non-negative-least-squares algorithm, yielding both the T_2 components present in the decay and their relative proportions. In addition, this procedure permitted an estimation of the water peak area at a theoretical TE of 0 ms (W_{TE0}). Metabolite and water MR signals and concentrations are related by the simple expression:

[Equation 1] $\quad \mathrm{WSB/WCB} = \mathrm{MSB/MCB}$

Where WSB is Water Signal from Brain, WCB is Water Concentration in Brain, MSB is Metabolite Signal from Brain, and MCB is Metabolite Concentration in Brain. Equation 1 can be rearranged to calculate metabolite concentration in brain:

[Equation 2] $\quad \mathrm{MCB} = \mathrm{WCB} \cdot \mathrm{MSB/WSB}$

Where WCB (in millimolar) is defined as:

[Equation 3] $\quad$ Pure water concentration (PWC) $= 1000 \cdot (1000/\mathrm{MW}_{water})$

Factoring in the respective water content for GM ($\mathrm{GM}_{water} = 0.80 \cdot \mathrm{PWC}$) and WM ($\mathrm{WM}_{water} = 0.65 \cdot \mathrm{PWC}$) gives:

[Equation 4] $\quad \mathrm{WCB} = \mathrm{W}_{brain} = (\mathrm{GM}_{segment} \cdot \mathrm{GM}_{water}) + (\mathrm{WM}_{segment} \cdot \mathrm{WM}_{water})$

After inserting the measured variables from equations 3 and 4 into equation 2 above we get:

[Equation 5] $MCB = [(W_{brain} \cdot M_{TE196})/ W_{TE0}]$

Allowing for different numbers of averages and metabolite T_2 values for metabolite and water acquisitions we define scaling factors (SF) as:

[Equation 6] $SF_{averages}$ = square root of $M_{averages}$ / square root of $W_{averages}$

and the SF for metabolite T_2 as:

[Equation 7] SF_{MT2} = exponential ($-TE / T_2$)

Finally, incorporating the scaling factors for equations 6 and 7 into equation 5 gives the expression for calculating the metabolite concentration in brain as:

[Equation 8] $MCB = SF_{T2} \cdot [(W_{brain} \cdot M_{TE196})/ W_{TE0}]/SF_{averages}$

The T_2 values for metabolites were assigned based on averaged literature values for NAA (350 msec), Cr (150 msec) and Cho (310 msec), and estimated for Glu (380 msec) based on expected normal brain concentration values for the GM:WM mix sampled in our studies. Also, the scaling factor accounting for metabolite T_2 is only to provide numerical values in the mM range, and the same values are applied to all data. This allows comparison to reported data. Metabolite concentrations expressed to brain water are reported in machine units and were compared using the Paired samples t-test, with significance evaluated at the $\alpha=0.05$ level, using SPSS© (version 11.0.4 for OS X 10.4.2).

5.3 Results

5.3.1 Pre-Clinical 18.8T ^{1}H NMR Spectroscopy Experiment

Figure 3.1 depicts a typical ^{1}H spectrum of rat brain extract acquired at 18.8T, with the DSS peak referenced to 0 ppm. At 18.8T, *myo*-inositol gives multiplet signals at 3.27, 3.52, 3.62, and 4.06 ppm. The spectral regions that contain contributions from *myo*-inositol are expanded.

5.3.1.1 High-Field NMR Spectroscopy Data

Metabolite concentrations are reported in μmol per gram of wet weight, normalized to the DSS standard concentration (Table 5.1). No significant differences between *myo*-inositol concentrations in pre-frontal and occipital cortex, and hippocampus were observed between vehicle- and dextro-amphetamine-treated rats (Independent samples t-test, $p>0.05$) (Table 5.1). There was a trend toward an increase in the temporal cortex in dextro-amphetamine treated rats, but this did not reach statistical significance (Independent samples t-test, $t=1.788$, 10 df, $p=0.104$).

Table 5.1: *myo*-Inositol Concentrations (μmol/gram wet weight) across Four Brain Regions, for dextro-Amphetamine and Vehicle-Treated Rats.

Region	**Treatment Group**	
	Vehicle	**Dextro-amphetamine**
Cortex		
Pre-Frontal[a]	2.20 ± 0.59	2.09 ± 0.44
Temporal	3.30 ± 0.40	5.17 ± 0.97
Occipital	3.26 ± 0.66	3.76 ± 0.27
Sub-Cortical		
Hippocampus	2.54 ± 0.10	3.28 ± 0.33

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Significance was assessed at $p<0.05$, using Independent Samples t-test.

[a] Five rats/group.

5.3.2 Clinical 3T ^{1}H MRS Experiment

5.3.2.1 Subject Characteristics

A total of 12 healthy volunteers (5 males, 7 females) who met the eligibility criteria participated in the present study. The mean age of the group was 29.50 years (± 1.30).

5.3.2.2 Physiological and Subjective Response

As anticipated from previous studies (Asghar et al. 2003; Willson et al. 2005), there was a significant dextro-amphetamine-induced increase in heart rate, systolic blood pressure and diastolic blood pressure (Table 5.2). The percentage change in VAS scores from baseline, for each item, are reported in Table 5.3. There was a main time effect on the change from baseline in the subjective measures of anxiety, happiness, energy, irritability, speed of thought and restlessness following dextro-amphetamine administration in healthy volunteers. Post-hoc analysis, in the form of paired sample t-tests, showed a significant increase from baseline to 75 min for all four items above, and from baseline to 150 min for five items above (Table 5.3). Physical restlessness and speed of thought both increased significantly across all three time-points. These results are consistent with dextro-amphetamine having been administered at a dose that causes its well-recognized psychological effects.

Table 5.2: Effects of Acute dextro-Amphetamine on Heart Rate and Blood Pressure.

Physiological Measure	Mean (SEM)
Heart Rate (beats per min), mean ± SEM [a]	
Baseline	64.3 ± 2.4
Pre-MRS (75 min)	75.0 ± 2.9
Post-MRS (150 min)	82.0 ± 2.2
Blood Pressure (mm Hg), mean ± SEM [b]	
Systolic	
Baseline	118.3 ± 2.6
Pre-MRS (75 min)	131.7 ± 2.5
Post-MRS (150 min)	134.2 ± 1.9
Diastolic	
Baseline	69.6 ± 2.6
Pre-MRS (75 min)	77.5 ± 2.0
Post-MRS (150 min)	76.2 ± 2.0

Measurements were taken at baseline, 75 min after dextro-amphetamine administration (Pre-MRS) and again at 150 min after dextro-amphetamine (Post-MRS).

[a] Heart rate significantly increased from Baseline to Post-MRS and Pre-MRS to Post-MRS, post-hoc paired samples t-test $p < 0.01$.

[b] Blood pressure, both systolic and diastolic, significantly increased from Baseline to Pre-MRS and Baseline to Post-MRS, post-hoc paired samples t-test $p < 0.05$.

Table 5.3: Effects ~~of~~ Acute dextro-Amphetamine on VAS Subjective Measures of Mood.

"0" Anchor	Mean ± SEM			"100" Anchor
	Baseline	75 min	150 min	
I don't feel anxious at all	20.50 ± 5.83[a]	39.25 ± 8.34	33.50 ± 7.91	I feel very anxious
I feel very miserable or sad	53.50 ± 9.56[b]	62.33 ± 8.58	71.83 ± 8.43	I feel very happy
I feel mentally slowed	63.17 ± 7.57[b]	78.75 ± 5.85	82.67 ± 5.31	I feel mentally alert
I feel physically unwell	78.50 ± 8.09	82.67 ± 5.48	82.00 ± 4.77	Physically I feel fine
I don't feel hungry at all	50.17 ± 6.86	42.67 ± 9.73	40.75 ± 7.47	I feel very hungry
I feel tired and lethargic	61.33 ± 6.50[b]	73.08 ± 6.83	84.42 ± 4.59	I feel very energetic
I can concentrate well	25.25 ± 7.84	38.92 ± 7.40	35.83 ± 10.22	I can't concentrate at all
I feel placid and calm	18.42 ± 4.94[a]	44.00 ± 5.60	36.67 ± 6.34	I feel very irritable
My thoughts are slow	46.50 ± 4.74[a,b]	67.75 ± 5.18[c]	80.67 ± 4.83	My thoughts are speeded up
I don't feel light headed	23.83 ± 7.68	47.25 ± 9.13	45.67 ± 9.93	I feel very light-headed
I feel physically inactive	44.67 ± 5.09[a,b]	61.92 ± 6.06[c]	73.08 ± 5.42	I feel physically restless

Measurements were taken at baseline, 75 min after dextro-amphetamine administration and again at 150 min after dextro-amphetamine. Scores could range from 0 to 100 (on a 100 mm scale) and are reported as Mean ± SEM.

[a] Significant increase from baseline to 75 min, post-hoc paired samples t-test $p < 0.05$.

[b] Significant increase from baseline to 150 min, post-hoc paired samples t-test $p < 0.05$.

[c] Significant increase from 75 min to 150 min, post-hoc paired samples t-test $p < 0.05$.

5.3.2.3 Clinical MRS Data

Analysis of the ^{1}H MRS data did not reveal any differences between pre- and post-dextro-amphetamine scans in terms of S/N ratios (mean ± SD: Pre = 20.3 ± 5.4; Post = 20.9 ± 4.2) nor in terms of the standard deviations of the fits for *myo*-inositol (Pre = 6.0% ± 1.5%; Post = 6.4% ± 0.7%), NAA (Pre = 2.8% ± 0.9%; Post = 2.8% ± 0.6%), Cr-PCr (Pre = 4.3% ± 1.5%; Post = 4.2% ± 0.7%), choline (Pre = 3.5% ± 1.0%; Post = 3.7% ± 0.5%), glutamate+glutamine (Pre = 14.2% ± 4.5%; Post = 13.3% ± 2.3%), or glutamate (Pre = 12.2% ± 4.1%; Post = 11.6% ± 1.6%) (all $p>0.400$). This indicates that the pre- and post-scans did not differ in quality. A representative ^{1}H MRS spectra from the DMPFC at 3T is shown in Figure 5.2, with LCModel fit. No differences in the distribution of GM, WM and CSF were found between pre and post dextro-amphetamine scans ($p>0.200$).

The use of an optimized sequence for *myo*-inositol acquired at 3T provided a well-resolved *myo*-inositol peak at ~3.6 ppm (Figure 5.2). No significant differences between the *myo*-inositol (m-Ino) was observed when comparing pre- with post-dextro-amphetamine values (Paired samples t-test, $t=-0.090$, 11 df, $p=0.930$) (Table 5.4).

Figure 5.2: A Representative ^{1}H MRS Spectra (black line) of Human DMPFC at 3T, with LCModel Fit (red line). The baseline is shown in blue.

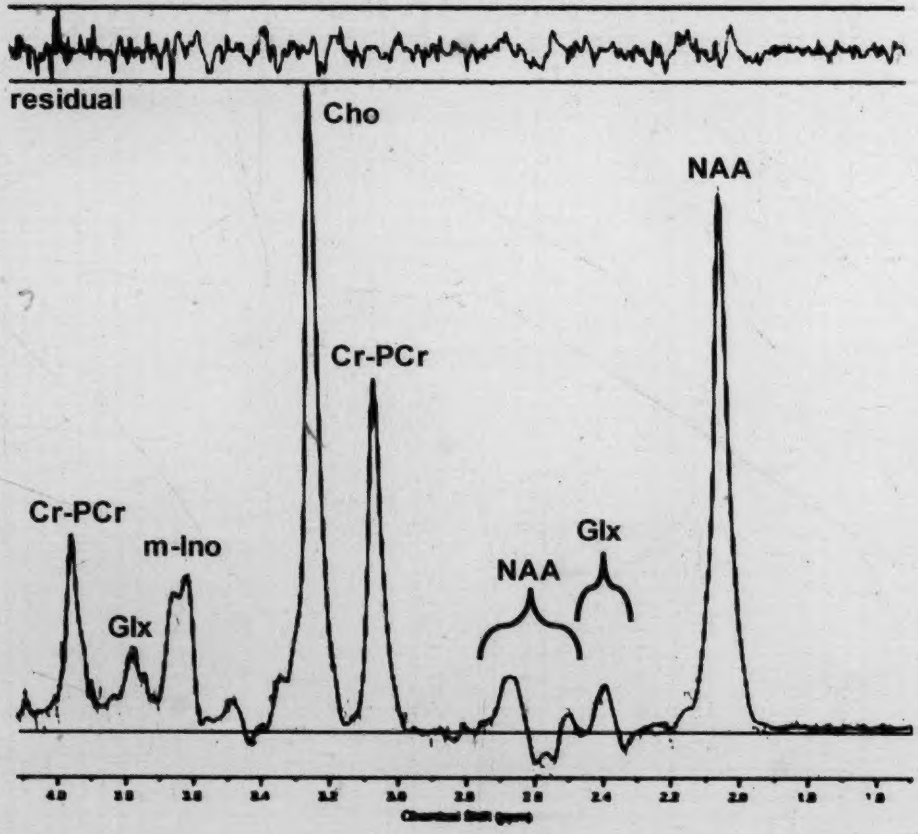


Table 5.4: 3T ^{1}H MRS *myo*-Inositol Concentrations for Baseline and Post dextro-Amphetamine Scans.

	Baseline	**Post d-Amphetamine**
Metabolite[a] m-Ino	1.10 ± 0.04	1.10 ± 0.06

Significance from baseline was assessed at $p<0.05$ using Paired Samples t-test.

[a] Concentration is reported in machine units (mean ± SEM), adjusted for grey matter fraction.

5.4 Discussion

The present set of experiments were designed to determine if acute administration of dextro-amphetamine would alter *myo*-inositol concentrations in either rats or humans. Such a finding would be of considerable clinical and experimental significance since it would link the similarity in subjective and physiological experiences of dextro-amphetamine users to bipolar mania with the purported involvement of the PI-cycle in BPD symptomatology. This would provide an important neurochemical model for the study of BPD pathophysiology. That said, the main finding in the present study was that *myo*-inositol concentrations were not altered *in vivo* by acute dextro-amphetamine administration in the DMPFC of HV nor in the frontal, temporal and occipital cortex, or in the hippocampus of rats.

These findings are in line with some of our earlier research, which showed no changes in whole rat brain (O'Donnell et al. 2000) or in HV (Silverstone et al. 1996, 1999). However, in another study our findings suggested that dextro-amphetamine increased phosphoinositol cycle activity in HV (Silverstone et al. 2002a). The present findings are more compelling for several reasons. Firstly, to overcome possible limitations in our previous animal studies, in the present study four brain regions were examined. Three of these regions (frontal and temporal cortex, and hippocampus) have been shown, clinically, to have altered PI-cycle functioning in BPD, while the forth region (occipital cortex) has not shown such alterations (reviewed in Silverstone et al. 2005). Furthermore, investigation of specific brain regions would reduce the likelihood of an averaging out effect with respect to any underlying neurochemical changes, an affect that may occur with the use of whole brain. Also, the magnet used in the present study is among the most powerful commercially available for *in vitro* use, providing highly resolved spectra. Rat brain has not been examined at 18.8T previously. The human volunteer study also differed from previous work via two fundamental improvements: namely, this study utilized a newly developed sequence specifically optimized for *myo*-inositol (Kim et al. 2005). This optimized sequence increased the stability and accuracy of the spectroscopy results via improvements in the signal-to-background and S/N, providing more precise measurement of brain *myo*-inositol. This study also reported *myo*-inositol concentrations in relation to brain water, not as a ratio to Cr-PCr or NAA, which have been shown to change (Hamakawa et al. 1999; Kato et al. 1995; Stanley et al. 2000). These improvements address major limitations present in the majority of past clinical MRS studies (reviewed in Silverstone et al. 2005).

Nonetheless, even with these methodological improvements and in contrast to both our initial hypotheses, the studies did not lead to detectable changes in *myo*-inositol concentrations between experimental and control conditions. Thus, from these results we conclude that while dextro-amphetamine does produce subjective and physiological effects similar to those observed in bipolar mania, it does not appear to induce changes in *myo*-inositol concentrations and presumably not in brain PI-cycle neurochemistry. While it is possible that the PI-cycle may be involved in the pathophysiology of bipolar disorder, it is not likely that the subjective and physiological of dextro-amphetamine are mediated, directly or indirectly, via this mechanism. Moreover, in rats dextro-amphetamine does not induce changes in *myo*-inositol concentrations in brain regions purported to be involved in mood regulation.

5.5 Bibliography

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nat New Biol 1971;233:267-268.

Anderzhanova E, Rayevsky KS, Saransaari P, Riitamaa E, Oja SS. Effects of sydnocarb and d-amphetamine on extracellular levels of amino acids in the rat caudate-putamen. Eur J Pharmacol 2001;428:87-95.

Anderzhanova E, Rayevsky KS, Saransaari P, Riitamaa E, Oja SS. Effects of acute toxic doses of psychostimulants on extracellular levels of excitatory amino acids and taurine in rats. Comparison of d-amphetamine and sydnocarb. Ann NY Acad Sci 2002;965:193-203.

Andiarzhanova EA, Saransaari R, Riitamaa E, Oiia SS, Raevskii KS. Extracellular level of neuroactive amino acids in the rat neostriatum after treatment with psychostimulants (microdialysis study). Eksp Klin Farmakol 2002;65:7-13.

Angrist B, Corwin J, Bartlik B, Cooper T. Early pharmacokinetics and clinical effects of oral dextroamphetamine in normal subjects. Biol Psychiatry 1987;22:1357-1368.

Asghar SJ, Tanay VA, Baker GB, Greenshaw A, Silverstone PH. Relationship of plasma amphetamine levels to physiological, subjective, cognitive and biochemical measures in healthy volunteers. Hum Psychopharmacol 2003;18:291-299.

Atack JR. Lithium, phosphatidylinositol signaling, and bipolar disorder: The role of inositol monophosphatase. In: Bipolar Medications – Mechanisms of Action. Manji HK, Bowden CL, Belmaker RH (eds.). Washington: American Psychiatric Press Inc.: Washington, DC. 2000.

Aylmer CG, Steinberg H, Webster RA. Hyperactivity induced by dexamphetamine/chlordiazepoxide mixtures in rats and its attenuation by lithium pretreatment: A role for dopamine? Psychopharmacol 1987;91:198-206.

Bearden CE, Hoffman KM, Cannon TD. The neuropsychology and neuroanatomy of bipolar affective disorder: A critical review. Bipolar Disord 2001;3:106-150.

Bell EC, Willson MC, Wilman AH, Dave S, Asghar SJ, Silverstone PH. Lithium and valproate attenuate dextroamphetamine-induced changes in brain activation. Human Psychopharmacol 2005;20:87-96.

Belmaker RH, Agam G, van Calker D, Richards MH, Kofman O. Behavioral reversal of lithium effects by four inositol isomers correlates perfectly with biochemical effects on PI cycle: Depletion by chronic lithium of brain inositol is specific to hypothalamus, and inositol levels may be abnormal in postmortem brain from bipolar patients. Neuropsychopharmacol 1998;19:220-232.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Berridge MJ, Irvine RF. Inositol phosphates and cell signaling. Nature 1989;341:197-205.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-917.

Bowden CL. Valproate. Bipolar Disord 2003;5:189-202.

Brauer LH, de Wit H. Subjective responses to dextroamphetamine alone and after pimozide pretreatment in normal, healthy volunteers. Biol Psychiatry 1996;39:26-32.

Bruhn H, Stoppe G, Staedt J, Merboldt KD, Hanicke W, Frahm J. Quantitative proton MRS in vivo shows cerebral myo-inositol and cholines to be unchanged in manic-depressive patients treated with lithium. In: Society of Magnetic Resonance in Medicine. New York, 1993, p. 1543.

Cappeliez P, Moore E. Effects of lithium on an amphetamine animal model of bipolar disorder. Prog Neuropsychopharmacol Biol Psychiatry 1990;14:347-358.

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children with familial bipolar disorder. Biol Psychiatry 2003;53:1059-1065.

Choe ES, Chung KT, Mao L, Wang JQ. Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors. Neuropsychopharmacol 2002;27:565-575.

Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M, McCracken J. Decreased anterior cingulated myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. Neuropsychopharmacol 2001;24:359-369.

Davanzo P, Yue K, Thomas MA, Belin T, Mintz J, Venkatraman TN, Santoro E, Barnett S, McCracken J. Proton magnetic resonance spectroscopy of bipolar disorder versus intermittent explosive disorder in children and adolescents. Am J Psychiatry 2003;160:1442-1452.

Del Arco A, Martinez R, Mora F. Amphetamine increases extracellular concentrations of glutamate in the prefrontal cortex of the awake rat: A microdialysis study. Neurochem Res 1998;23:1153-1158.

Drevets WC. Neuroimaging and neuropathological studies of depression: Implications for the cognitive-emotional features of mood disorders. Cur Opin Neurobiol 2001;11:240-249.

Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in the nervous system: An update. J Neurochem 1992;58:18-38.

Folstein MF, Luria R. Reliability, validity, and clinical application of the Visual Analogue Mood Scale. Psychol Med 1973;3:479-486.

Frey R, Metzler D, Fischer P, Heiden A, Scharfetter J, Moser E, Kasper S. Myo-inositol in depressive and healthy subjects determined by frontal 1H-magnetic resonance spectroscopy at 1.5 tesla. J Psychiatr Res 1998;32:411-420.

Gani D, Downes CP, Batty I, Bramham J. Lithium and myo-inositol homeostasis. Biochim Biophys Acta 1993;1177:253-269.

Geddes JR, Burgess S, Hawton K, Jamison K, Goodwin GM. Long-term lithium therapy for bipolar disorder: Systematic review and meta-analysis of randomized controlled trials. Am J Psychiatry 2004;161:217-222.

Goodwin FK, Jamison KR. Manic Depressive Illness. Oxford University Press: New York, NY. 1990.

Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 1993;29:804–811.

Hamakawa H, Kato T, Shioiri T, Inubushi T, Kato, N. Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. Psychol Med 1999;29:639-644.

Hanstock CC, Allen PS. Segmentation of brain from a PRESS localised single volume using double inversion recovery for simultaneous T1 nulling. Proceedings of the 8th Annual Meeting of the International Society of Magnetic Resonance in Medicine, Denver, USA, 2000.

Hanstock CC, Coupland NJ, Allen PS. GABA X_2 multiplet measured pre- and post-administration of vigabatrin in human brain. Magn Reson Med 2002;48:617–623.

Herman E. Lamotrigine: A depression mood stabilizer. Eur Neuropsychopharmacol 2004;14:S89-S93.

Hoebel BG, Hernandez L, Schwartz DH, Mark GP, Hunter GA. Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. Ann NY Acad Sci 1989;575:171-191.

Jacobs D, Silverstone T. Dextroamphetamine induced arousal in human subjects as a model for mania. Psychol Med 1986;16:323-329.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded ^{31}P-MRS. Psychol Med 1995;25:557-566.

Kato T, Shioiri T, Takahashi S, Inubushi T. Measurement of brain phosphoinositide metabolism in bipolar patients using in vivo ^{31}P-MRS. J Affect Disord 1991;22:185-190.

Kato T, Takahashi S, Shioiri T, Inubushi T. Alterations in brain phosphorus metabolism in bipolar detected by in vivo ^{31}P and ^{7}Li magnetic resonance spectroscopy. J Affect Disord 1993;27:53-60.

Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. J Affect Disord 1994;31:125-133.

Kim H, Thompson RB, Hanstock CC, Allen PS. Variability of metabolite yield using STEAM or PRESS sequences in vivo at 3.0 T, illustrated with myo-inositol. Magn Reson Med 2005;53:760-769.

Markley JL, Bax A, Arata Y, Hilbers CW, Kaptein R, Sykes BD, Wright PE, Wuthrich K. Recommendations for the presentation of NMR structures of proteins and nucleic acids. IUPAC-IUBMB-IUPAB Inter-Union Task Group on the Standardization of Data Bases of Protein and Nucleic Acid Structures Determined by NMR Spectroscopy. J Biomol NMR 1998;12:1-23.

McElroy SL, Keck PE Jr., Strakowski SM. Mania, psychosis and antipsychotics. J Clin Psychiatry 1996;57:14-26.

Mcgeehan AJ, Janak PH, Olive MF. Effect of the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) on the acute locomotor stimulant properties of cocaine, D-amphetamine, and the dopamine reuptake inhibitor GBR12909 in mice. Psychopharmacol 2004;174:266-273.

McGrath BM, Greenshaw AJ, McKay R, Hanstock CC, Seres P, Slupsky CM, Weljie AM, Dave S, Silverstone PH. Dextro-amphetamine is not a PI-cycle based model of mania: Data from clinical (3T) and preclinical (18.8T) MRS studies. Proceedings of the 61st Annual Meeting of the Society of Biological Psychiatry. Biol Psychiatry 2006;59(8, Suppl 1):52S.

Moore GJ, Galloway MP. Magnetic resonance spectroscopy: Neurochemistry and treatment effects in affective disorders. Psychopharmacol Bull 2002;36:5-23.

Mora F, Porras A. Effects of amphetamine on the release of excitatory amino acid neurotransmitters in the basal ganglia of the conscious rat. Can J Physiol Pharmacol 1993;71:348-351.

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH. Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 2000;880:84-91.

Pellegrino LJ, Pellegrino AS, Cushman AJ. A Stereotaxic Atlas of the Rat Brain, 2nd Edition. Plenum Press: New York, NY. 1979.

Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672–679.

Redpath TW, Smith FW. Technical note: Use of a double inversion recovery pulse sequence to image selectively grey or white brain matter. Br J Radiol 1994;67:1258–1263.

Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: Effects on catecholamine systems and behavior. Ann Rev Pharmacol Toxicol 1993;33:639-677.

Sharma R, Venkatasubramanian PN, Barany M, Davis JM. Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizophr Res 1992;8:43-49.

Silverstone PH, McGrath BM, Wessels PH, Bell EC, Ulrich M. Current pathophysiological findings in bipolar disorder and in its subtypes. Curr Psychiatry Rev 2005;1:75-101.

Silverstone PH, O'Donnell T, Ulrich M, Asghar S, Hanstock CC. Dextroamphetamine increases phosphoinositol cycle activity in volunteers: An MRS study. Human Psychopharmacol Clin Exp 2002a;17:425-429.

Silverstone PH, Pukhovsky A, Rotzinger S. Lithium does not attenuate the effects of dextroamphetamine in healthy volunteers. Psychiatry Res 1998;79:219-226.

Silverstone PH, Rotzinger S, Pukhovsky A, Hanstock CC. Effects of lithium and amphetamine on inositol metabolism in human brain as measured by ^{1}H and ^{31}P MRS. Biol Psychiatry 1999;46:1634-1641.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Hum Psychopharmacol Clin Exp 2002b;17:321-327.

Slupsky CM. The NMR Solution Structure of Calcium-Saturated Skeletal Muscle Troponin C., in Thesis: Biochemistry. 1995, University of Alberta: Edmonton. p. 352.

Stanley JA, Pettegrew JW, Keshavan MS. Magnetic resonance spectroscopy in schizophrenia: Methodological issues and findings – part I. Biol Psychiatry 2000;48:357-368.

Tohen M, Ketter TA, Zarate CA, Suppes T, Frye M, Altshuler L, Zajecka J, Schuh LM, Risser RC, Brown E, Baker RW. Olanzapine versus divalproex sodium for the treatment of acute mania and maintenance of remission: A 47-week study. Am J Psychiatry 2003;160:1263-1271.

Weisler RH, Kalali AH, Ketter TA. A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of Extended-Release Carbamazepine Capsules as Monotherapy for Bipolar Disorder Patients with Manic or Mixed Episodes. J Clin Psychiatry 2004;65:478-484.

Williams RS, Cheng L, Mudge AW, Harwood AJ. A common mechanism of action for three mood-stabilizing drugs. Nature 2002;417:292-295.

Wüthrich K. NMR of Proteins and Nucleic Acids. John Wiley & Sons: New York, NY. 1986.

Yildiz A, Demopulos CM, Moore CM, Renshaw PF, Sachs GS. Effect of lithium on phosphoinositide metabolism in human brain: A proton decoupled (31)P magnetic resonance spectroscopy study. Biol Psychiatry 2001;50:3-7.

Chapter 6. *myo*-Inositol Metabolism in Bipolar-II and Unipolar Depression.

(A version of this chapter has been presented at the 61st annual meeting of the Society of Biological Psychiatry – see McGrath et al. 2006a)

6.1 Introduction

Mood disorders are among the leading causes of morbidity in humans worldwide (Murray and Lopez 1996), and are the most common severe psychiatric disorders of adult onset (Craddock and Forty 2006). Current classification envisions two main categories of mood disorders, UPD and BPD (American Psychiatric Association 2000). The requirement for the occurrence of at least one episode of mania (BPD-I) or hypomania (BPD-II) during the course of the illness distinguishes BPD from UPD, in which individuals only experience episodes of depression. Unipolar major depression is about five to ten times more common than BPD (Simpson et al. 2002; Weissman et al. 1988; Kessler et al. 1994; Spaner et al. 1994).

Among the symptoms of BPD, patients experience depressive symptoms far more frequently than they do manic or hypomanic symptoms (Judd et al. 2002; Judd et al. 2003; Post et al. 2003). In one of the most recent studies to assess this, Judd and associates (2002, 2003) noted that BPD-I patients were depressed for about 32% of the follow-up period, while BPD-II patients spent about 50% of this time with depressive symptoms. This inequality in symptom presentation is also likely the primary reason why as many 25% of patients with BPD (particularly BPD-II) are misdiagnosed as suffering from UPD at initial assessment (Hantouche et al. 1998). Evidence also exists to suggest that BPD patients are at an increased risk of suicide (up to 30 times) during periods of depression, compared to periods of mania or hypomania (Kalin 1996/97; Compton and Nemeroff 2000). Given this, and the observation that patients spend only about 9% and 2% of the time with manic or hypomanic symptoms, respectively, a better understanding of the pathophysiology of depression is required in order for improvements in the treatment and management of patients with BPD to be made.

Considering its prevalence and severity, the pathophysiological underpinnings that underlie depressive symptomatology are not fully understood. Much of the clinical research into depression has focused mainly on patients with the UPD variant, often building on findings from the effects of antidepressant medication on preclinical models of the disorder. From a neuroanatomical perspective, the frontal brain region is of particular interest to the study of depressive pathophysiology, given that this region plays a key role in processes that control mood and cognitive function (Fuster 2002; Heilman and Gilmore 1998). More specifically, the ventromedial prefrontal region has been associated with emotional experience (Rolls 1999; Steele and Lawrie 2004), while the dorsolateral region has been linked to cognitive function (Vogt et al. 1992; Fuster 1997) in both humans and animals. Neurosurgical approaches to treatment-resistant mood disorders have even targeted these regions (Dougherty et al. 2003; Ebmeier et al. 2006). Also, the monoamine neurotransmitter systems all have projections from subcortical ganglia, including the dorsal raphe and the locus ceruleus, to regions in the frontal cortex. The fMRI literature has shown abnormal activity, across several paradigms, in frontal brain regions among depressed patients relative to HC (reviewed in Silverstone et al. 2005). More specifically, studies have shown increased activity in emotion-related frontal regions and decreased activity in cognitive-related frontal regions (Mayberg 2003; Steele and Lawrie 2004; Drevets 2000). Anatomical MRI studies have also reported volumetric reductions in the frontal cortex of patients with mood disorders (Anand and Shekhar 2003; Haldane and Frangou 2004; Silverstone et al. 2005).

In recent years, human MRS studies have added to our understanding of the pathophysiology of mood disorders. In particularly, several studies have examined the role of *myo*-inositol in BPD pathophysiology, across symptom states (Table 2.1 – 2.3). *Myo*-inositol is of particular interest in BPD, as the most widely examined neurochemical hypothesis for this disorder has been the inositol depletion hypothesis. This hypothesis was formulated based on early findings in the preclinical literature that lithium inhibits turnover of the PI-cycle in an uncompetitive manner via inhibition of IMPase and inositolpolyphosphate 1-phosphatase, resulting in a reduction in *myo*-inositol concentration (Allison and Stewart 1971; Berridge and Irvine 1989; Berridge et al. 1982). In neurons, the PI-cycle has been found to be activated following ligand binding with G_q-protein coupled receptors, including adrenergic (α_{1A} and α_{1B}), dopaminergic (D_1), serotonergic (5-HT_{1C} and 5-HT_2), and cholinergic (M_1 and M_3) receptor subtypes among others (Fisher et al. 1992) (Figure 1.2). Because of this inhibition, Berridge and colleagues (1982) proposed that lithium-induced decreases in *myo*-inositol concentrations are the basis for lithium's mechanism of therapeutic action. This inhibition attenuates the usual neuronal responses to receptor activation, since there is less *myo*-inositol with which to produce IP_3. Because there is less IP_3 generated, the rise in internal calcium concentration that would otherwise have occurred is prevented, attenuating downstream cellular responses. In the clinical milieu, this inhibition can be detected by measuring changes in *myo*-inositol concentration using MRS.

In considering all published studies to date, the evidence from depressed BPD patients suggests that in both medicated and unmedicated patients there may be a decrease in *myo*-inositol concentrations in the frontal region, with less support for changes in other regions (reviewed in Silverstone et al. 2005). Postmortem evidence from suicide victims and bipolar patients is suggestive of reduced frontal cortex *myo*-inositol (Shimon et al. 1997), but MRS studies in depressed UPD patients have been inconsistent (reviewed in Coupland et al. 2005). It is plausible that depressed UPD patients also have altered PI-cycle functioning given that several serotonergic and noradrenergic receptors are linked to this second messenger system, the fact that augmenting mood stabilizer treatment with antidepressant medication has been shown to be effective for depressed BPD-II patients (Amsterdam and Brunswick 2003; Bowden 2005), and the shift in the conceptualization of these disorders, from that of a unipolar-bipolar dichotomy to that of a bipolar spectrum with similar or related underlying pathophysiologies (Lara and Akiskal 2006; Akiskal and Pinto 1999). Much of the inconsistency in past MRS studies of BPD and UPD depression has been due, in part, to several methodological limitations. Firstly, the majority of studies have employed a 1.5T magnet to collect data on *myo*-inositol. Magnets of this field strength have limited spectral resolution, when compared to higher field strengths such as 3T (Figure 1.3). Moreover, most measurements of *myo*-inositol have assessed changes in the multiplet resonating at 3.56 – 3.65 ppm (or ~3.6 ppm). The spins of this multiplet are strongly coupled (Cerdan et al. 1985; Govindaraju et al. 2000), causing the signal to degrade as the echo time is increased due to *J* evolution and spin-spin relaxation (Kim et al. 2005). Shorter echo times can be employed (Pouwels and Frahm 1998; Brooks et al. 1999; Zhong and Ernst 2004); however, this increases the signal contribution of both the macromolecules (Behar et al. 1994) and neighbouring metabolites including glutamate, glutamine and taurine (Kim et al. 2005), making it more difficult to resolve the spectral contribution of *myo*-inositol. Secondly, studies to date have quantified *myo*-inositol concentrations as a ratio to another detectable brain metabolite, most often creatine-phosphocreatine or *N*-acetylaspartate. This approach has been based on the important assumption that the denominator is unchanging, which is likely not the case. Finally, most studies that have examined *myo*-inositol have done so using standard spectral acquisition parameters, not specifically tailored for the detection of *myo*-inositol. This is compounded by the proximity of the *myo*-inositol peak to the large water peak – which can interfere if not sufficiently suppressed – and to the overlapping resonance of glycine at 3.55 ppm. Due to the above, studies reporting results for *myo*-inositol have been inconsistent. Moreover, it is still unclear whether there are differences in *myo*-inositol concentrations between the BPD subtypes and whether or not BPD and UPD depressed patients have differences in PI-cycle functioning.

Thus, the present study improves upon those limitations outlined above and aims at testing the hypothesis that *myo*-inositol concentrations will be altered in depressed BPD-II depressed patients relative to age- and sex-matched depressed UPD patients and HC subjects. Furthermore, the present study will assess differences, if any, in *myo*-inositol concentrations between depressed UPD patients and age- and sex-matched HC subjects. This study employs a novel MRS acquisition methodology specifically designed to measure *myo*-inositol concentrations in the human brain at 3T (Kim et al. 2005). To this author's knowledge, this is the first clinical MRS study to specially examine *myo*-inositol levels in depressed BPD-II patients and to compare these with levels in depressed UPD patients.

6.2 Materials and Methods

6.2.1 Subjects

This study was approved by the local human research ethics board of the University of Alberta Hospital, and all participants gave full informed consent. Subject recruitment began in the fall of 2004 and ended in the summer of 2006. Patients with BPD-II depression and UPD depression were recruited from the Bipolar Referral Clinic at the University of Alberta Hospital. This clinic provides family physicians with a rapid referral process for patients suspected of having a mood disorder. Patients are typically seen by a psychiatrist within a few weeks of referral. A full personal and family history was obtained and diagnosis was made using the Diagnostic and Statistical Manual of Mental Disorders (version IV-TR) by a licensed psychiatrist. At the time of the scan, all patients were also interviewed by the author (BMM) using the SCID (First et al. 1997). Patients were also assessed on the global assessment of functioning scale (GAF) (Endicott et al. 1976; American Psychiatric Association 2000), 17-item HAM-D (Hamilton 1960), the MADRS (Montgomery and Asberg 1979) and the YMRS (Young et al. 1978). Patients were excluded if they did not meet DSM-IV criteria for depression, presently met the DSM-IV criteria for mania or hypomania, had another Axis I disorder (excluding anxiety), had a recent history (<1 year) of serious substance abuse, had a significant neurological disease, including seizures, were pregnant or lactating within the last 6 months, or had any contraindications to magnetic resonance scans.

Healthy controls were recruited through a newsletter advertisement from the university community and matched to patients based on age and sex. Controls also underwent a brief medical history, and a detailed SCID to rule out past or present psychiatric illness. Controls were also assessed on the GAF, HAM-D, MADRS and the YMRS. Controls were excluded if they presently had any Axis I disorder (including anxiety), were treated for a Axis I disorder in the past, had any history of Axis I disorders in first-degree relatives, had a recent history (<1 year) of serious substance abuse, had a significant neurological disease, including seizures, were pregnant or lactating within the last 6 months, or had any contraindications to magnetic resonance scans.

6.2.2 ^{1}H MRS

Data were acquired with a 3.0T scanner (Magnex Scientific, Abingdon, United Kingdom/Surrey Medical Imaging Systems, Guilford, United Kingdom) with a 28-cm quadrature birdcage resonator for transmission and reception. Multi-slice gradient echo images (TR = 500 msec, TE = 22 msec, slice thickness = 5 mm, 11 slices, resolution = 256 × 256) were used to co-register a 2.5 x 2.5 x 2.5-cm PRESS voxel that encompassed DMPFC. The lower border was orientated to a line connecting the anterior and posterior commissures. The voxel was centered on the midline, touching the tip of the genu of the corpus callosum (Figure 5.1). The PRESS-selected volume was used for both tissue segmentation and for acquiring water-suppressed metabolite spectra. Shimming was carried out first with FASTMAP (Gruetter 1993) to optimize the linear and non-linear shims over a 5-cm-diameter spherical volume, then with an automatic optimization of the linear *x, y, z* shims on the PRESS-selected voxel at TE1 = 36 msec, TE2 = 160 msec and TR = 3000 msec. Typical shimmed water line-widths were better than

6 Hz (0.05 ppm). The PRESS acquisitions were performed as the sum of 16 sub-spectra, each of 32 averages, allowing re-registration to the same frequency reference (the acetyl peak of NAA at 2.023 ppm) to eliminate the effects of frequency drift during the course of the acquisition.

6.2.2.1 Tissue Segmentation

Tissue segmentation into GM, WM, and CSF was performed with a double inversion recovery sequence to acquire 1D projections of each T_1 compartment in the PRESS-selected volume (Hanstock and Allen 2000). Two hyperbolic secant inversion pulses (110-msec length, bandwidth = 150 Hz) were added to the PRESS pulse sequence, in which the pulses were 90° sinc-gauss and 180° optimized-sinc shapes. Before the 90° pulse, a 15-msec spoiler gradient was applied to dephase any transverse magnetization resulting from the inversion pulses. The PRESS parameters were TR = 9 sec, TE = 120 msec, two averages with 5-kHz sample frequency, digitized over 128 data points. Prior data were used to estimate the T_1 values for the three brain compartments (GM: 1070 ± 60 msec; WM: 720 ± 30 msec; CSF: 4440 ± 50 msec). With the expression derived by Redpath and Smith (1994), two pairs of T_{inv1} and T_{inv2} timings were computed that gave signal nulls of the CSF compartment with either that from either GM or WM. Twenty-one 1D projections were acquired, with ten projections for each set of double-null inversion timings, and one with just CSF nulled. An additional 10 1D projections were acquired with no inversion pulses and with a TE of 500 msec. This minimized the signal contamination from GM and WM (<0.2% residual signal after accounting for T_2 losses), while maintaining significant signal from CSF (approximately 50% residual signal).

After phase correction of each projection, three-dimensional surface and contour maps were generated to confirm that the selected T_{inv1} and T_{inv2} timings were resulting in simultaneous nulls of CSF with either GM or WM. Subsequently, GM and WM projections were selected from the double inversion recovery 1D projection series. The T_{inv1} and T_{inv2} timings that were used to acquire these two series of projections for either GM or WM were then used to estimate a normalization factor that reflected attenuation due to T_1 and T_2 losses for each projection, thereby fully accounting for all the acquisition timings. Similarly, a normalization factor was estimated for the CSF projection. Segmentation resulted from first normalizing and then summing the signal across each of the three projections, such that the relative proportions of GM, WM, and CSF could be estimated. These proportions were then used to calculate a grey matter fraction [GM/(GM + WM)] for the voxel. All computations were performed within the MATLAB program environment (MathWorks, Natick, Massachusetts).

6.2.2.2 Identification and Quantification of Brain Metabolites

Internal water data used as the reference MR signal standard for metabolite quantification were acquired using STEAM sequence at different TE values (TR=12 sec, TM=30 msec). Three STEAM acquisition series were performed, each as the sum of 2 averages for each TE value, for a total of 18 different TE values. Echo times in the first acquisition ranged from 20 msec – 100 msec, with a step size of 20 msec. Echo times in the second acquisition ranged from 150 msec – 500 msec, with a step size of 50 msec. Echo times in the third acquisition ranged from 700 msec – 1500 msec, with a step size of 200 msec.

Metabolite quantification was achieved using the signal from brain water, and by utilizing the following three series of data. These included: metabolite (M) peak area estimates, extracted from LCModel program (Provencher 1993) output (M_{TE196}); segmentation information for GM, WM and CSF compartment sizes, used to estimate water (W) concentration in the selected brain voxel (W_{brain}), and; internal water data acquired at different TE values used as the reference MR signal standard (W_{TE0}).

Metabolite spectra were Fourier transformed and analyzed with LCModel. The basis spectra for LCModel analysis were simulated within the spectral bandwidth of 1.00–3.90 ppm with numeric methods (Hanstock et al. 2002). The metabolites included in the basis spectra were NAA, *N*-acetylaspartyl-glutamate, Cr, choline, glutamate, glutamine, GABA, *myo*-inositol, glycine, taurine, and aspartate. The in vivo metabolite data were accepted if the S/N ratio was 11 or more, and the standard deviation of the fit for the metabolite was $\leq$20%. In the present study, no subjects were excluded based on the aforementioned criteria.

For quantification, the water data were first imported into the processing software, and filtered, Fourier transformed; phase and baseline corrected. The water peak area from each spectrum in the TE series was determined and these area data were fitted to a multi-exponential using a non-negative-least-squares algorithm, yielding both the T_2 components present in the decay and their relative proportions. In addition, this procedure permitted an estimation of the water peak area at a theoretical TE of 0 ms (W_{TE0}). Metabolite and water MR signals and concentrations are related by the simple expression:

[Equation 1] $\quad WSB/WCB = MSB/MCB$

Equation 1 can be rearranged to calculate metabolite concentration in brain:

[Equation 2] $\quad MCB = WCB \cdot MSB/WSB$

Where WCB (in millimolar) is defined as:

[Equation 3] $\quad PWC = 1000 \cdot (1000/MW_{water})$

Factoring in the respective water content for GM ($GM_{water} = 0.80 \cdot PWC$) and WM ($WM_{water} = 0.65 \cdot PWC$) gives:

[Equation 4] $\quad WCB = W_{brain} = (GM_{segment} \cdot GM_{water}) + (WM_{segment} \cdot WM_{water})$

After inserting the measured variables from equations 3 and 4 into equation 2 above we get:

[Equation 5] $MCB = [(W_{brain} \cdot M_{TE196})/ W_{TE0}]$

Allowing for different numbers of averages and metabolite T_2 values for metabolite and water acquisitions we define SF as:

[Equation 6] $SF_{averages}$ = square root of $M_{averages}$ / square root of $W_{averages}$
and the SF for metabolite T_2 as:

[Equation 7] SF_{MT2} = exponential (-TE / T_2)

Finally, incorporating the scaling factors for equations 6 and 7 into equation 5 gives the expression for calculating the metabolite concentration in brain as:

[Equation 8] $MCB = SF_{T2} \cdot [(W_{brain} \cdot M_{TE196})/ W_{TE0}]/SF_{averages}$

The T_2 values for metabolites were assigned based on averaged literature values for NAA (350 msec), Cr (150 msec) and Cho (310 msec), and estimated for Glu (380 msec) based on expected normal brain concentration values for the GM:WM mix sampled in our studies. Also, the scaling factor accounting for metabolite T_2 is only to provide numerical values in the mM range, and the same values are applied to all data. This allows comparison to reported data.

6.2.3 Statistical Analysis

Metabolite concentrations (in machine units) were compared using a one-way ANOVA, with significance evaluated at the α=0.05 level. Post hoc comparisons were conducted using Tukey's HSD test. Correlations were carried out using Pearson's bivariate correlation coefficient. Age, sex and family history of mood disorders were found not to be significant covariants, and analysis was collapsed across these variables. GM fraction was also found not to be a significant covariant with metabolite concentrations. All data were analyzed with SPSS© (version 11.0.4 for OS X 10.4.2).

6.3 Results

6.3.1 Subject Characteristics

A total of 9 depressed BPD-II patients, 16 depressed UPD patients and 19 healthy controls met the eligibility criteria and participated in the present study. Spectroscopy data for 3 BPD-II and 4 UPD patients had to be excluded due to poor data quality resulting from computational errors during water quantification. These patients did not differ from the remaining patients in terms of their age, sex, GAF, HAM-D or YMRS score (Mann-Whitney U, $p>0.100$). Thus, data from 6 depressed BPD-II patients, 12 depressed UPD patients and 19 healthy controls were included in the present analysis. Demographic and clinical information for these participants is presented in table 6.1.

Groups did not differ on age [$F(2,34)=1.961$, $p=0.156$] or sex [$F(2,34)=1.1426$, $p=0.254$]. Both patient groups had significantly lower GAF scores relative to HC (post hoc Tukey HSD, $p<0.001$). Both patient groups also had significantly higher HAM-D (post hoc Tukey HSD, $p<0.001$), MADRS (post hoc Tukey HSD, $p<0.001$) and YMRS (post hoc Tukey HSD, $p<0.005$) mean total scores relative to HC. Differences between patients and controls in YMRS scores were primarily contributable to differences in the item assessing sleep (item 4). Scores on the HAM-D and MADRS were strongly correlated (Pearson $r=0.939$, $p<0.001$). BPD and UPD patients differed statistically only on their YMRS mean total score, with bipolar depressed patients having a higher score on this scale (post hoc Tukey HSD, $p<0.05$). This difference is contributable to the item assessing increases in motor activity and energy (item 2) and the item assessing increases in speech rate and amount (item 6), for both of which bipolar patients scored significantly higher. Of the 6 BPD patients, 5 were taking some form of psychiatric medication, with 4 patients taking more than one type of medication. Among the 5 medicated patients, 1 was taking divalproex sodium at the time of the scan, 1 was prescribed an antipsychotic, and 5 were prescribed the antidepressant venlafaxine. Of the 12 unipolar patients, 9 were taking some form of psychiatric medication, with 7 patients taking more than one type of medication. Among these 9, 3 were taking an antipsychotic, and 8 were taking an antidepressant, of which venlafaxine was the most common. No patients were prescribed lithium. All healthy controls had a negative history for taking psychiatric medication.

Table 6.1: Patient and Volunteer Demographic and Clinical Characteristics.

	BPD-II (n=6)	**UPD (n=12)**	**HC (n=19)**
Age (y)	28 (7)	42 (15)	34 (16)
Sex (female/male)	5/1	10/2	11/8
HAM-D[a]	17 (7)	18 (7)	1 (1)
MADRS[a]	22 (8)	24 (8)	1 (2)
YMRS[b,c]	6 (6)	3 (1)	0.5 (1)
GAF[a]	58 (5)	56 (7)	99 (2)
Medication (n)	5	9	All were medication-free
Mood Stabilizer	1	0	
Antidepressant	5	8	
Antipsychotic	1	3	
Polypharmacy	4	7	

Scores are reported as Mean (SD).

[a] $p<0.001$, [b] $p<0.005$: patients compared to healthy controls.

[c] $p<0.05$: BPD-II compared to UPD patients.

6.3.2 ^{1}H MRS Data

6.3.2.1 Data Quality

Analysis of the ^{1}H MRS data did not reveal any differences between the three groups in terms of S/N ratios (mean ± SD: BPD-II = 19.3 ± 5.9; UPD = 19.0 ± 4.5; HC = 20.1 ± 5.2) nor in terms of the standard deviations of the fits for *myo*-inositol (BPD-II = 6.5% ± 1.0%; UPD = 6.2% ± 1.3%; HC = 6.0% ± 1.3%), *N*-acetylaspartate (BPD-II = 3.3% ± 0.8%; UPD = 2.8% ± 0.7%; HC = 2.9% ± 0.8%), creatine-phosphocreatine (BPD-II = 4.7% ± 1.2%; UPD = 4.3% ± 0.8%; HC = 4.2% ± 1.2%), choline (BPD-II = 3.7% ± 0.8%; UPD = 3.4% ± 0.8%; HC = 3.5% ± 0.8%), glutamate+glutamine (BPD-II = 12.3% ± 2.4%; UPD = 13.9% ± 3.4%; HC = 15.0% ± 5.7%), or glutamate (BPD-II = 11.0% ± 2.4%; UPD = 11.8% ± 1.7%; HC = 12.8% ± 3.9%) (all $p>0.400$). This indicates that group differences are not a result of variability in scan quality. Representative ^{1}H MRS spectra from the DMPFC of depressed BPD-II patients, depressed UPD patients and HC subjects acquired at 3T are shown in Figure 6.1 – 6.3, with LCModel fit. No differences in the distribution of GM, WM and CSF were found between groups ($p>0.900$).

Figure 6.1: A Representative ^{1}H MRS Spectrum (black line) of the DMPFC from a BPD-II Depressed Patient, with LCModel fit (red line). The baseline is shown in blue.

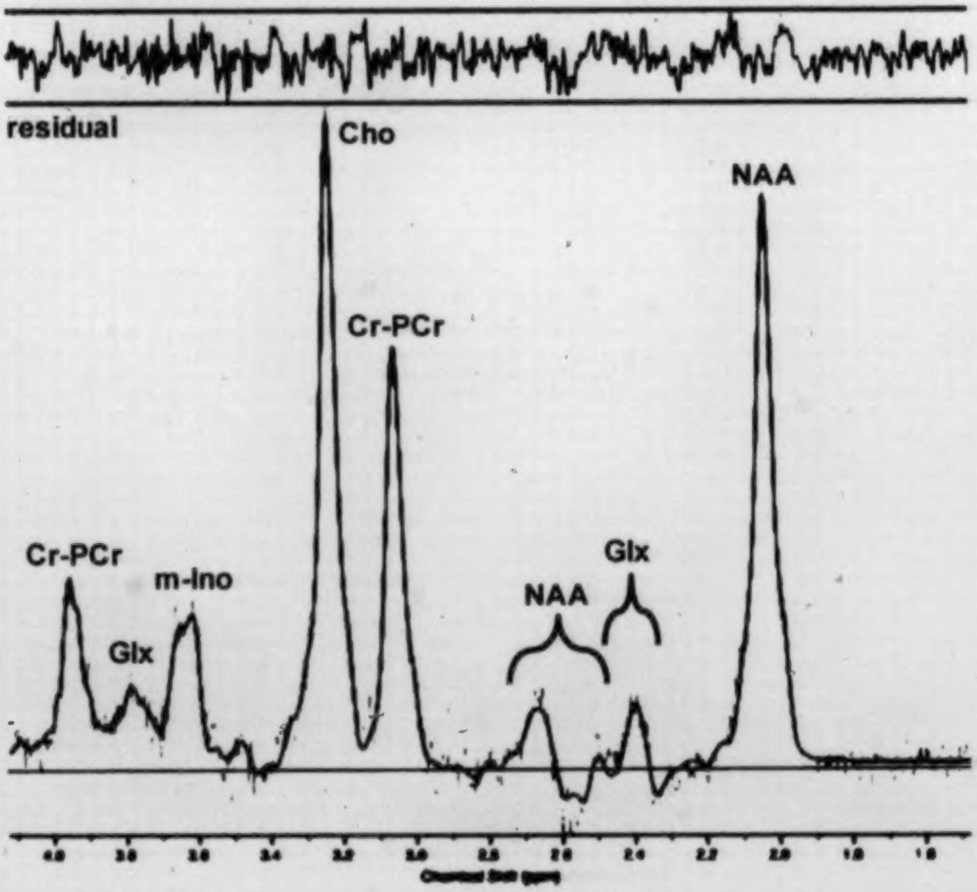


Figure 6.2: A Representative ^{1}H MRS Spectrum (black line) of the DMPFC from a UPD Depressed Patient, with LCModel fit (red line). The baseline is shown in blue.

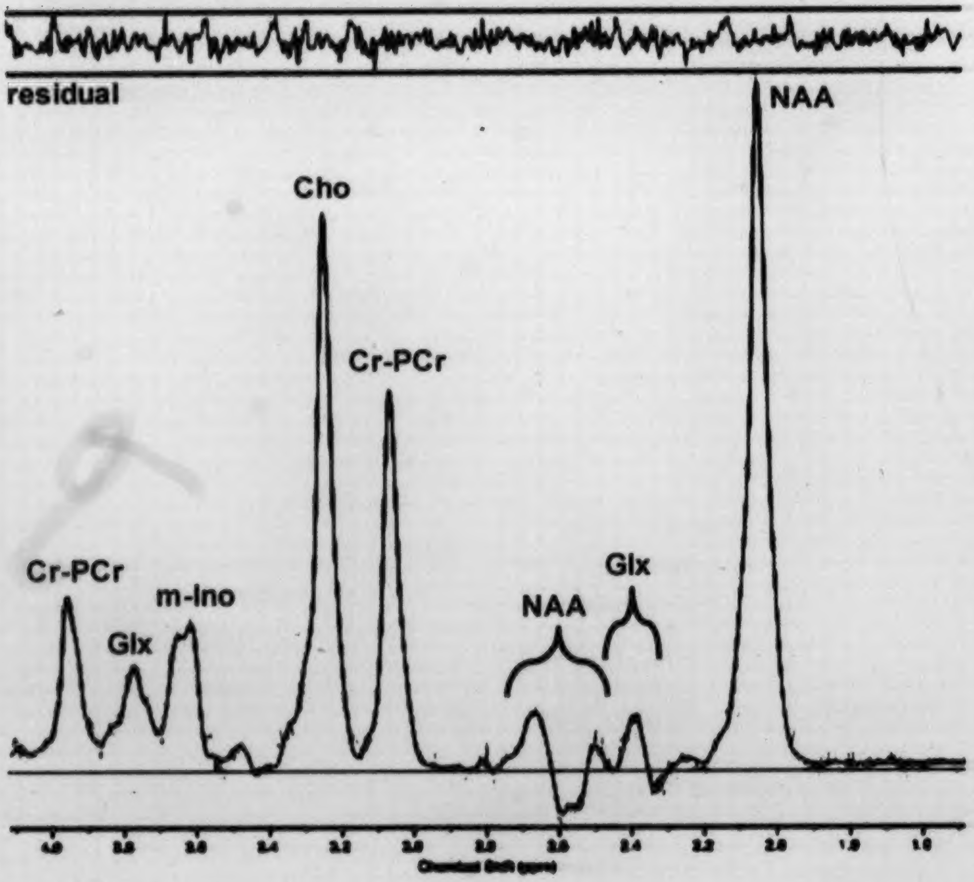
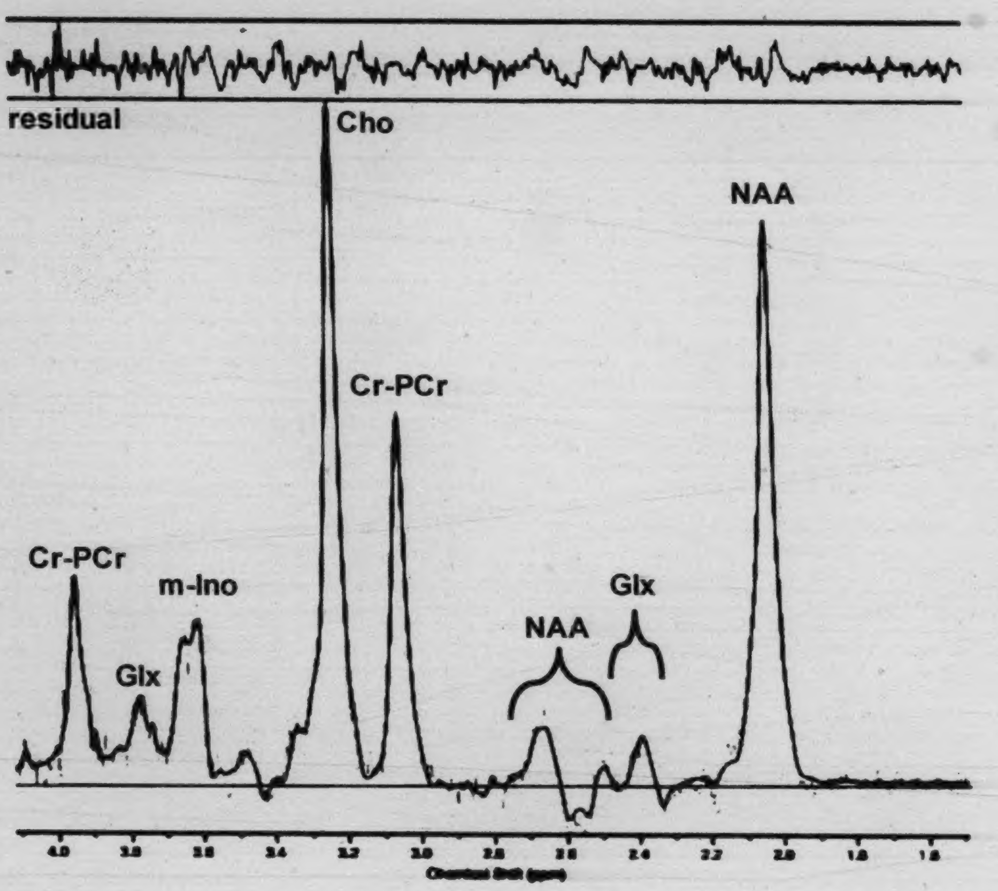


Figure 6.3: A Representative ^{1}H MRS Spectrum (black line) of the DMPFC from a HC Subject, with LCModel fit (red line). The baseline is shown in blue.



6.3.2.2 myo-Inositol (m-Ino)

The use of an optimized sequence for m-Ino acquired at 3T provided a well-resolved m-Ino peak at ~3.6 ppm (Figure 6.1 – 6.3). No significant differences between m-Ino concentrations were observed when comparing depressed BPD-II patients, depressed UPD patients and HC subjects (F(2,34)=1.742, p=0.190). There was a non-significant trend toward increased m-Ino concentration in the BPD-II group (by ~16% of HC values), and in the UPD group (by ~8% of HC values), relative to HC (Table 6.2 and Figure 6.4). There was no relationship between m-Ino concentration and patient's medication status (Pearson r=-0.223, p=0.213). There was a significant positive correlation between YMRS scores and m-Ino concentrations (Pearson r=0.330, p=0.046). No relationship existed between scores on the HAM-D and MADRS and m-Ino concentrations (p>0.050).

6.3.2.3 N-Acetylasparate (NAA)

The concentration of NAA did not differ statistically between depressed BPD-II patients, depressed UPD patients and HC subjects (F(2,34)=0.516, p=0.602) (Table 6.2 and Figure 6.5). There was no relationship between NAA concentrations and patient's medication status, nor between their scores on the HAM-D, MADRS and YMRS (p>0.600).

6.3.2.4 Choline (Cho)

Similarly, the three groups did not differ significantly in terms of their Cho concentrations (F(2,34)=2.927, p=0.067) (Table 6.2 and Figure 6.6). There was also a non-significant trend toward an increase in Cho concentrations in depressed BPD-II patients (by ~21% of HC values) and depressed UPD patients (by ~12% of HC values), relative to HC subjects. Similar to the above, Cho concentrations did not correlate significantly with patient's medication status (Pearson r=-0.262, p=0.141). There was a significant positive correlation between HAM-D scores and Cho concentrations (Pearson r=0.399, p=0.015). No relationship existed between scores on the YMRS and MADRS and Cho concentrations (p>0.050).

6.3.2.5 Creatine-Phosphocreatine (Cr-PCr)

The three groups also did not differ significantly in terms of their Cr-PCr concentrations (F(2,34)=2.866, p=0.071) (Table 6.2 and Figure 6.6). Again, there was a non-significant trend toward an increase in Cr-PCr concentrations in depressed BPD-II patients (by ~18% of HC values) and depressed UPD patients (by ~12% of HC values), relative to HC subjects. Similar to the above, Cr-PCr concentration did not correlate significantly with patient's medication status (Pearson r=-0.281, p=0.113). There was a significant positive correlation between Cr-PCr concentrations and both scores on the HAM-D (Pearson r=0.444, p=0.006) and MADRS (Pearson r=0.338, p=0.041), and a non-significant trend for scores on the YMRS (Pearson r=0.287, p=0.085).

Table 6.2: 3T ^{1}H MRS Metabolite Concentrations for BPD-II Depressed Patients, UPD Depressed Patients and HC Subjects.

	BPD-II (n=6)	**UPD (n=12)**	**HC (n=19)**
Metabolites[a]			
m-Ino	1.30 ± 0.09	1.19 ± 0.11	1.09 ± 0.03
NAA	1.30 ± 0.15	1.43 ± 0.09	1.35 ± 0.05
Cho	0.42 ± 0.03	0.39 ± 0.03	0.35 ± 0.01
Cr-PCr	0.96 ± 0.06	0.91 ± 0.07	0.82 ± 0.01

[a] Reported as ratios to brain water in machine units (mean ± SEM), adjusted for grey matter fraction.

Figure 6.4: Differences between BPD-II Depressed Patients, UPD Depressed Patients and HC Subjects in DMPFC *myo*-Inositol Concentrations (mean ± SEM) at 3T.

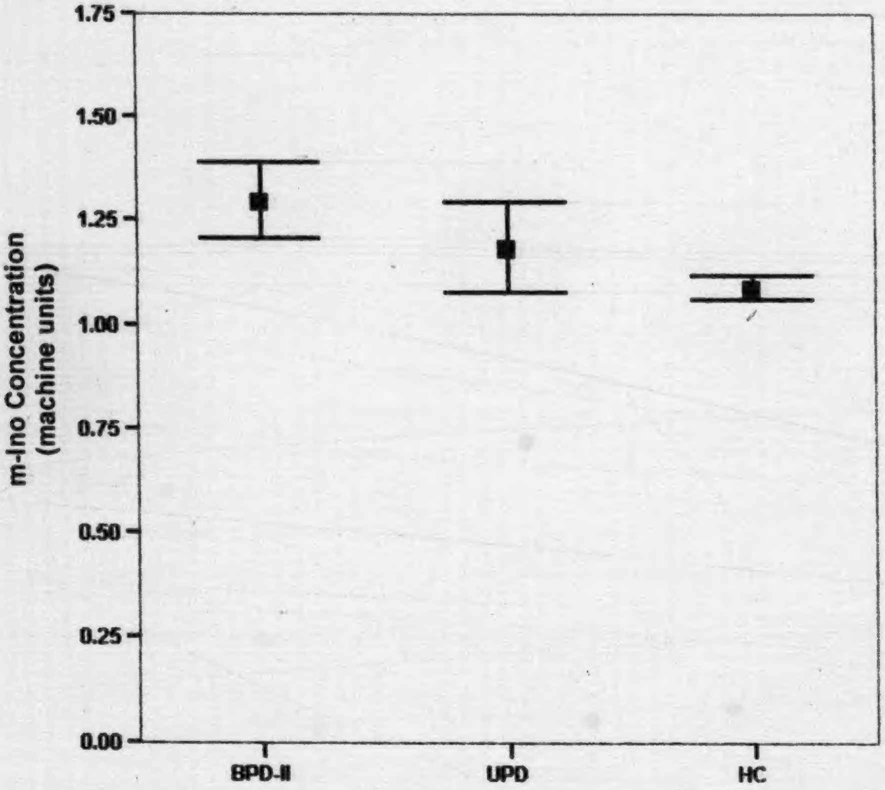


Figure 6.5: Differences between BPD-II Depressed Patients, UPD Depressed Patients and HC Subjects in DMPFC *N*-Acetylasparate Concentrations (mean ± SEM) at 3T.

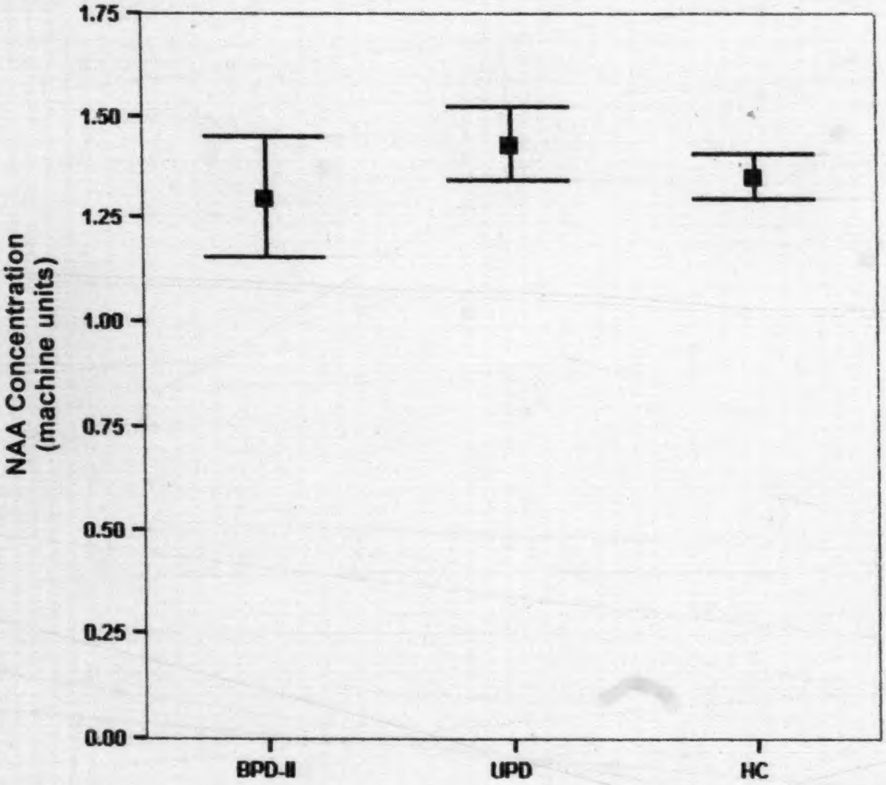


Figure 6.6: Differences between BPD-II Depressed Patients, UPD Depressed Patients and HC Subjects in DMPFC Choline Concentrations (mean ± SEM) at 3T.

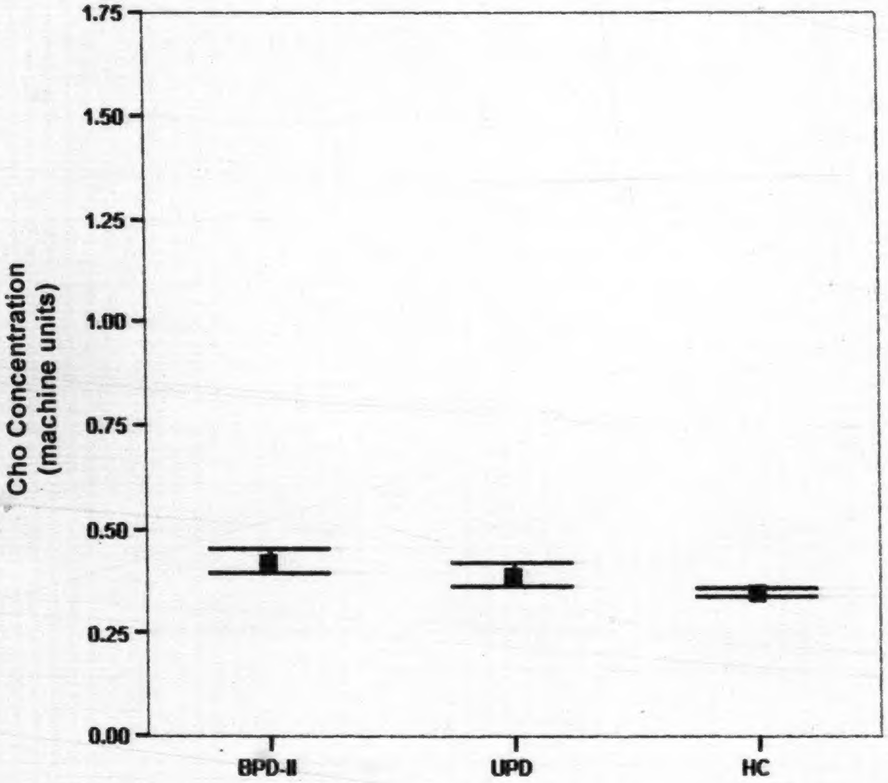
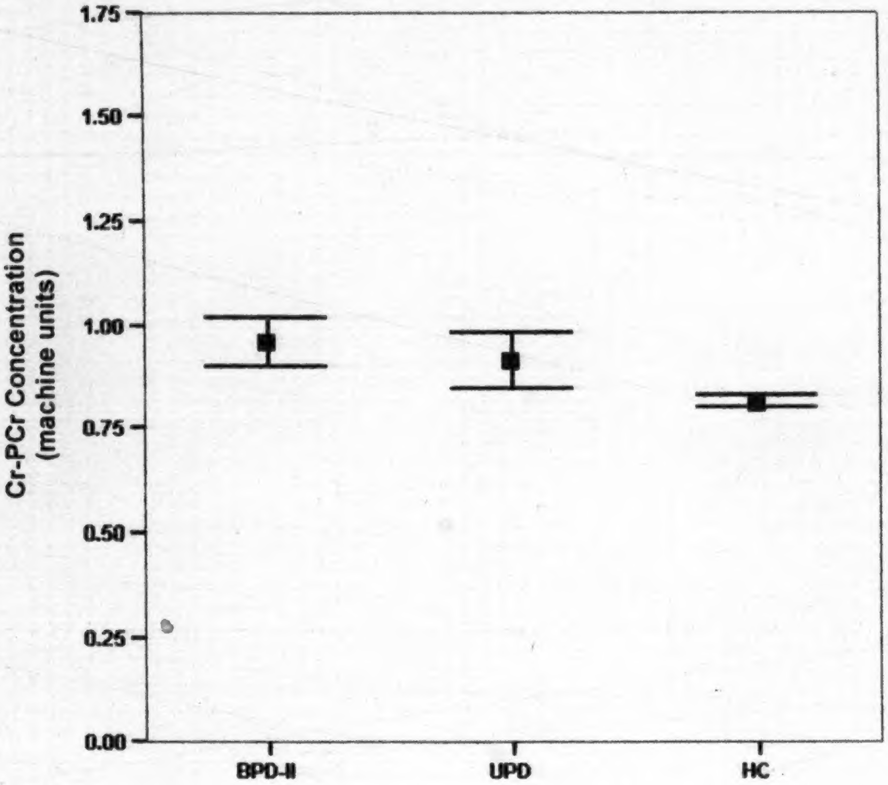


Figure 6.7: Differences between BPD-II Depressed Patients), UPD Depressed Patients and HC Subjects in DMPFC Creatine-Phosphocreatine Concentrations (mean ± SEM) at 3T.



6.4 Discussion

The main finding in the present study was that *myo*-inositol concentrations were not significantly altered in the DMPFC of depressed BPD-II patients relative to age- and sex-matched depressed UPD patients and HC subjects. A non-significant trend did exist toward an increase in DMPFC *myo*-inositol concentrations among BPD-II patients, relative to both UPD patients and HC. Interestingly, this trend is in the same direction as the *myo*-inositol alterations reported in manic BPD patients (reviewed in Silverstone et al. 2005). Moreover, it is opposite to the effects induced by lithium (McGrath et al. 2006b). This is interesting, in that although depressive and manic symptoms occupy opposite ends of the mood spectrum, the biochemical basis for these symptoms may be similar. This is supported by the fact that lithium (Yatham et al. 1997; Yatham et al. 2005), and as reported more recently, the antipsychotic olanzapine (Berk et al. 1999; Tohen et al. 2003) both show efficacy in the treatment of manic and depressive symptoms. Further support for this idea comes from the observation that *myo*-inositol concentrations in the depressed UPD group were midway between those of depressed BPD-II patients and HC subjects, in line with the idea of a mood disorders spectrum (Akiskal and Pinto 1999). It is unclear from the literature whether or not depressed BPD-I patients would have higher *myo*-inositol concentrations relative to patients with BPD-II. That said the *myo*-inositol concentrations for the patient groups in the present study had greater variability than those observed in the HC subjects and any differences in *myo*-inositol concentrations may be secondary to some other pathological change(s) in the brains of these patients. This requires further clarification. While interesting, the differences in *myo*-inositol concentrations were not significant and may be of little pathophysiological significance to either BPD-II or UPD.

No significant differences were found for DMPFC *N*-acetylaspartate, creatine-phosphocreatine and choline concentrations between depressed BPD-II patients, depressed UPD patients and HC subjects. *N*-Acetylaspartate is the most readily detectable neurometabolite by MRS, and as such comprises the largest peak, besides water, that is visible in standard ^{1}H-MRS at clinical field strengths. *N*-Acetylaspartate is found predominantly in neurons and axons and is purported to be involved in synthetic processes within these structures, and has been suggested as a marker of neuronal integrity (Malhi et al. 2002). In psychiatric disorders, decreased *N*-acetylaspartate has been proposed to reflect neuronal degeneration (Tsai and Coyle 1995), and this may be related to findings of volumetric reductions in the frontal brain region of patients with BPD (Drevets et al. 1997; Sax et al. 1999; Lyoo et al. 2004). Findings from the MRS literature examining *N*-acetylaspartate in BPD and UPD have not been consistent, with some studies finding reductions (Chang et al. 2003; Winsberg et al. 2000; Cecil et al. 2002; Gallelli et al. 2005), others increases (Dager et al. 2004; Sharma et al. 1992), and still others no concentration differences from HC levels (Silverstone et al. 2002; Bertolino et al. 2003; Castillo et al. 2000; Davanzo et al. 2001). There is also evidence to suggest that *N*-acetylaspartate increases in BPD patients who respond to treatment versus those who do not (DelBello et al. 2006).

Choline concentrations showed a pattern similar to that of *myo*-inositol, with BPD-II having the highest concentrations and HC subjects with the lowest, but not significantly so. A significant positive correlation was found between choline concentrations and scores on the HAM-D, but not the other scales. In biological systems, choline is an important component of cell membranes, and is a precursor of neurotransmitter acetylcholine. As such, choline may be implicated in the pathophysiology of mood disorders through several avenues, including increased cholinergic activity (Janowsky et al. 1972), alterations in phosphatidylcholine-linked signal transduction (Exton 1994), choline-induced changes in oxidative metabolism (Duc et al. 1997), and endocrine function (Gupta et al. 1995). While the majority of previous MRS studies of this neurometabolite in bipolar disorder have also not shown differences between patients and controls, one study did find that anterior cingulate choline concentrations positively correlated with patient's score on the HAM-D (Moore et al. 2000). This relationship had not been observed in other brain regions until now.

Again, similar to the above, creatine-phosphocreatine concentrations did not differ significantly between the three groups, although a trend toward an increase in BPD-II and UPD patients was observed. Creatine and phosphocreatine are involved in cellular energy metabolism. The relationship between the relative concentrations of these two metabolites is dependent on the energy demands of the cell, with phosphocreatine being the means of storing a high energy phosphate, which when released to form ATP results in the formation of creatine (Moore and Galloway 2002). It has been argued that abnormalities in concentrations of creatine and phosphocreatine may indicate hypometabolism, possibly as a result of mitochondrial dysfunction (Stork and Renshaw 2005). Of all the neurometabolites readily detectable at clinical field strengths, these metabolites have received the least direct investigation across most of the psychiatric literature. This has largely been the result of the fact that these metabolites are most often used as the ratio to which other metabolites are quantified and compared, with the assumption that they themselves are unchanging. Of the 9 studies to date that have evaluated creatine+phosphocreatine concentrations in bipolar disorder, 7 found no differences between patients and controls across several brain regions and mood states (Hamakawa et al. 1998; Hamakawa et al. 1999; Cecil et al. 2002; Michael et al. 2003; Friedman et al. 2004; Dager et al. 2004; Brambilla et al. 2005a). The findings in UPD are similar (Brambilla et al. 2005b). Although the present study did not find a statistical difference, the ~18% difference in creatine+phosphocreatine concentrations between BPD-II patients and HC lends further support to the notion that using the creatine+phosphocreatine ratio approach to quantification may be ill advised and biased. The use of newer quantification methodologies that are not dependent on creatine+phosphocreatine will allow more in-depth, hypothesis-driven studies of this important neurometabolite.

The present study made two fundamental improvements over previous MRS research in bipolar disorder. First, this study utilized a newly developed sequence specifically optimized for *myo*-inositol (Kim et al. 2005). This optimized PRESS sequence increased the stability and accuracy of the spectroscopy results via improvements in the signal-to-background and S/N, providing more precise measurement of brain *myo*-inositol. This study also reported *myo*-inositol concentrations in relation to brain water, not as a ratio to creatine+phosphocreatine or *N*-acetylaspartate, which changed in past studies (Hamakawa et al. 1999; Kato et al. 1995; Stanley et al. 2000). Also, the present study found a large difference between patient and control levels of this metabolite. This finding calls to question much of the early work using ratio quantification approaches. Both of these improvements address major limitations present in the majority of past clinical MRS studies (reviewed in Silverstone et al. 2005).

The results of the present study must be appraised in the context of the study's limitations. The main limitation of the present study was that not all patients were medication-free at the time of scan. Treatment effects have been reported to change metabolite concentrations; however, most of the evidence has been for lithium and no patients in the present study were treated with this mood stabilizer. Although many of the patients in the present study were receiving treatment (primarily antidepressants) for a mood disorder, they were also clinically depressed at the time of the scan. As such, it could be inferred that treatment was either not yet effective, or had discontinued being so. Similarly, if treatment acts by correcting symptom-producing physiological alterations, symptom return may be indicative of a return of such alterations. As such, treatment could be expected to be a greater confound in patients who have recovered and are currently not experiencing symptoms, which would be indicative of effective and successful treatment, and less of a confound in those who while currently treated are still symptomatic. The latter situation may indicate that the treatment is having no physiological effect relevant to the disorder it is intended to treat. Nevertheless, a treatment effect cannot be ruled out. Secondly, the BPD-II group had only 6 patients who were used in the final analysis. Power analysis has shown that this number is adequate to detect changes in *myo*-inositol in the range of 15% to 20% (C Hanstock, personal communication); however, caution must be exercised in drawing any firm inferences from the present results for the BPD-II group.

Considering both the methodological improvements and limitations, results from the present study did not find any differences between depressed BPD-II and UPD patients and HC, but are suggestive of a *myo*-inositol increase in BPD-II depression consistent with that of bipolar mania. It is less clear if this metabolite is implicated in UPD. This study also confirms that expressing metabolites as a ratio to creatine+phosphocreatine is not a reliable approach, and should be avoided in future studies of this nature.

6.5 Bibliography

Akiskal HS, Pinto O. The evolving bipolar spectrum. Prototypes I, II, II and IV. Psychiatric Clin N Am 1999;22:517-534.

Allison JH, Stewart MA. Reduced brain inositol in lithium-treated rats. Nat New Biol 1971;233:267-268.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revised. American Psychiatric Publishing Group: New York, NY. 2000.

Amsterdam JD, Brunswick DJ. Antidepressant monotherapy for bipolar type II major depression. Bipolar Disord 2003;5:388-395.

Anand A, Shekhar A. Brain imaging studies in mood and anxiety disorder: Special emphasis on the amygdala. Ann NY Acad Sci 2003;985:370-388.

Behar K, Rothman DL, Spencer DD, Petroff OAC. Analysis of macromolecule resonances in 1H NMR spectra of human brain. Magn Reson Med 1994;32:294-302.

Berk M, Ichim L, Brook S. Olanzapine compared to lithium in mania: A double-blind randomized controlled trial. Int Clin Psychopharmacol 1999;14:339-343.

Berridge MJ, Downes CP, Hanley MR. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 1982;206:587-595.

Berridge MJ, Irvine RF. Inositol phosphates and cell signaling. Nature 1989;341:197-205.

Bertolino A, Frye M, Callicott JH, Mattay VS, Rakow R, Shelton-Repella J, Post R, Weinberger DR. Neuronal pathology in the hippocampal area of patients with bipolar disorder: A study with proton magnetic resonance spectroscopic imaging. Biol Psychiatry 2003;53:906-913.

Bowden CL. Valproate. Bipolar Disord 2003;5:189-202.

Bowden CL, Ketter TA, Sashs GS, Thase ME. Focus on bipolar disorder treatment. J Clin Psychiatry 2005;66:1598-1609.

Brambilla P, Stanley JA, Nicoletti MA, Sassi RB, Mallinger AG, Frank E, Kupfer D, Keshavan MS, Soares JC. ^{1}H magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in bipolar disorder patients. J Affect Disord 2005a;86:61-67.

Brambilla P, Stanley JA, Nicoletti MA, Sassi RB, Mallinger AG, Frank E, Kupfer D, Keshavan MS, Soares JC. ^{1}H magnetic resonance spectroscopy study of dorsolateral prefrontal cortex in unipolar mood disorder patients. Psychiatry Res 2005b;138:131-139.

Brooks JCW, Roberts N, Kemp GJ, Martin PA, Whitehouse GH. Magnetic resonance imaging-based compartmentation and its application to measuring metabolite concentrations in the frontal lobe. Magn Reson Med 1999;41:883-888.

Castillo M, Kwock L, Courvoisie H, Hooper SR. Proton MR spectroscopy in children with bipolar affective disorder: Preliminary observations. Am J Neuroradiol 2000;21:832-838.

Cecil KM, DelBello MP, Moray R, Strakowski SM. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord 2002;4:357-365.

Cerdan S, Parrilla R, Santoro J, Rico M. ^{1}H NMR detection of cerebral myo-inositol. FEBS Lett 1985;187:167-172.

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children with familial bipolar disorder. Biol Psychiatry 2003;53:1059-1065.

Compton MT, Nemeroff CB. The treatment of bipolar depression. J Clin Psychiatry 2000;61:57-67.

Coupland NJ, Ogilvie CJ, Hegadoren KM, Seres P, Hanstock CC, Allen PS. Decreased prefrontal myo-inositol in major depressive disorder. Biol Psychiatry 2005;57:1526-1534.

Craddock N, Forty L. Genetics of affective (mood) disorders. Eur J Hum Genetics 2006;14:660-668.

Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Brain metabolic alterations in medication-free patients with bipolar disorder. Arch Gen Psychiatry 2004;61:450-458.

Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M, McCracken J. Decreased anterior cingulated myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. Neuropsychopharmacol 2001;24:359-369.

Del Arco A, Martinez R, Mora F. Amphetamine increases extracellular concentrations of glutamate in the prefrontal cortex of the awake rat: A microdialysis study. Neurochem Res 1998;23:1153-1158.

DelBello MP, Cecil KM, Adler CM, Daniels JP, Strakowski SM. Neurochemical effects of olanzapine in first-hospitalization manic adolescents: A proton magnetic resonance spectroscopy study. Neuropsychopharmacol 2006;31:1264-1273.

Dougherty DD, Weiss AP, Cosgrove GR, Alpert NM, Cassem EH, Nierenberg AA, Price BH, Mayberg HS, Fischman AJ, Rauch SL. Cerebral metabolic correlates as potential predictors of response to anterior cingulotomy for treatment of major depression. J Neurosurg 2003;99:1010-1017.

Drevets WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. In: Progress in Brain Research. Uylings HBM, Van Eden CG, De Bruin JPC, Feenstra MGP, Pennartz CMA (eds.). Elsevier Science: London, UK. 2000.

Drevets WC, Price JL, Simpson Jr, JR, Todd RD, Reich T, Vannier M, Raichle ME. Subgenual prefrontal cortex abnormalities in mood disorders. Nature 1997;386:824-827.

Duc CO, Weber Ah, Trabesinger AH, Meier D, Boesiger P. Recycling the cholines. Int Soc Magn Reson Med 1997;6:1210.

Ebmeier KP, Donaghey C, Steele JD. Recent developments and current controversies in depression. Lancet 2006;367:153-167.

Endicott J, Spitzer RL, Fleiss JL, Cohen J. The global assessment scale. A procedure of measuring overall severity of psychiatric disturbance. Arch Gen Psychiatry 1976;33:766-771.

Exton JH. Phosphatidylcholine breakdown and signal transduction. Biochem Biophys Acta 1994;1212:26-42.

First MB, Williams BW, Spitzer RL. Structured clinical interview for the DSM-IV Axis I psychiatric disorders. American Psychiatric Press: Washington, DC. 1997.

Fisher SK, Heacock AM, Agranoff BW. Inositol lipids and signal transduction in the nervous system: An update. J Neurochem 1992;58:18-38.

Friedman SD, Dager SR, Parow A, Hirashima F, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Lithium and valproic acid treatment effects on brain chemistry in bipolar disorder. Biol Psychiatry 2004;56:340-348.

Fuster JM. Frontal lobe and cognitive development. J Neurocytol 2002;31:373-385.

Fuster JM. The Prefrontal Cortex. Lippincott-Raven: New York, NY. 1997.

Galleli KA, Wagner CM, Karchemskiy A, Howe M, Spielman D, Reiss A, Chang KD. N-acetylaspartate levels in bipolar offspring with and at high-risk for bipolar disorder. Bipolar Disord 2005;7:589-597.

Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed 2000;13:129-153.

Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 1993;29:804–811.

Gupta RK, Bhatia V, Poptani H, Gujral RB. Brain metabolic changes on in vivo proton magnetic resonance spectroscopy in children with congenital hypothyroidism. J Pediatr 1995;126:389-392.

Haldane M, Frangou S. New insights help define the pathophysiology of bipolar affective disorder: Neuroimaging and neuropathology findings. Prog Neuropsychopharm Biol Psychiatry 2004;28:943-960.

Hamakawa H, Kato T, Murashita J, Kato N. Quantitative proton magnetic resonance spectroscopy of the basal ganglia in patients with affective disorders. Eur Arch Psychiatr Clin Neurosci 1998;248:53-58.

Hamakawa H, Kato T, Shioiri T, Inubushi T, Kato, N. Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. Psychol Med 1999;29:639-644.

Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;23:56-62.

Hanstock CC, Allen PS. Segmentation of brain from a PRESS localised single volume using double inversion recovery for simultaneous T1 nulling. Proceedings of the 8th Annual Meeting of the International Society of Magnetic Resonance in Medicine, Denver, USA, 2000.

Hanstock CC, Coupland NJ, Allen PS. GABA X_2 multiplet measured pre- and post-administration of vigabatrin in human brain. Magn Reson Med 2002;48:617–623.

Hantouche EG, Akiskal HS, Lancrenon S, Allilair JF, Sechter D, Azorin JM, Bourgeois M, Fraud JP, Chatenet-Duchene L. Systematic clinical methodology for validating bipolar-II disorder: Data in mid-stream from a French multi-site study (EPIDEP). J Affect Disord 1998;50:163-173.

Heilman KM, Gilmore RL. Cortical influences in emotion. J Clin Neurophysiol 1998;15:409-423.

Janowsky DS, El Yousef MK, Dario JM. A cholinergic adrenergic hypothesis of mania and depression. Lancet 1972;1:1385-1386.

Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon DA, Leon AC, Rice JA, Keller MB. The long-term natural history of the weekly symptomatic status of bipolar I disorder. Arch Gen Psychiatry 2002;59:530-537.

Judd LL, Akiskal HS, Schettler PJ, Coryell W, Endicott J, Maser J, Solomon DA, Leon AC, Keller MB. A prospective investigation of the natural history of the long-term weekly symptomatic status of bipolar II disorder. Arch Gen Psychiatry 2003;60:261-269.

Kalin NH. Management of the depressive component of bipolar disorder. Depress Anxiety 1996/97;4:190-198.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded ^{31}P-MRS. Psychol Med 1995;25:557-566.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: Results from the national comorbidity survey. Arch Gen Psychiatry 1994;51:8-19.

Kim H, Thompson RB, Hanstock CC, Allen PS. Variability of metabolite yield using STEAM or PRESS sequences in vivo at 3.0 T, illustrated with myo-inositol. Magn Reson Med 2005;53:760-769.

Lara DR, Akiskal HS. Toward an integrative model of the spectrum of mood, behavioral and personality disorders based on fear and anger traits: II. Implications for neurobiology, genetics and psychopharmacological treatment. J Affect Disord 2006; Epub ahead of print.

Lyoo IK, Kim MJ, Stoll AL, Demopulos CM, Parow AM, Dager SR, Friedman SD, Dunner DL, Renshaw PF. Frontal lobe gray matter density decreases in bipolar I disorder. Biol Psychiatry 2004;55:648-651.

Malhi GS, Valenzuela M, Wen W, Sachidev P. Magnetic resonance spectroscopy and its applications in psychiatry. Aust NZ J Psychiatry 2002;36:31-43.

Mayberg HS. Modulating dysfunctional limbic-cortical circuits in depression: Towards development of brain-based algorithms for diagnosis and optimized treatment. In: Imaging Neuroscience: Clinical Frontiers for Diagnosis and Management. Frackowiak RS, Jones T (eds.). Oxford University Press: Oxford, UK. 2003.

McGrath BM, Greenshaw AJ, McKay R, Slupsky CM, Silverstone PH. Lithium alters regional rat brain myo-inositol at 2 and 4 weeks: An ex vivo MRS study at 18.8 Tesla. NeuroReport 2006b.

McGrath BM, Hanstock CC, Seres P, Dave S, Silverstone PH. Phosphinositol metabolism in depression: Is there a difference between bipolar and unipolar disorders? Proceedings of the 61st Annual Meeting of the Society of Biological Psychiatry. Biol Psychiatry, 2006a;59(8, Suppl 1):136S.

Michael N, Erfurth A, Ohrmann P, Gossling M, Arolt V, Heindel W, . Acute mania is accompanied by elevated glutamate/glutamine levels within the left dorsolateral prefrontal cortex. Psychopharmacol 2003;168:344-346.

Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:383-389.

Moore CM, Breeze JL, Gruber SA, Babb SM, Frederick BB, Villafuerte RA, Stoll AL, Hennen J, Yurgelum-Todd DA, Cohen BM, Renshaw PF. Choline, myo-inositol and mood in bipolar disorder: A proton magnetic resonance spectroscopic imaging study of the anterior cingulated cortex. Bipolar Disord 2000;2:207-216.

Moore GJ, Galloway MP. Magnetic resonance spectroscopy: Neurochemistry and treatment effects in affective disorders. Psychopharmacol Bulletin 2002;36:5-23.

Murray CJL, Lopez AD (eds.). The global burden of disease: A comprehensive assessment of mortality, injuries, and risk factors in 1990 and projected to 2020. Harvard School of Public Health: Cambridge, MA. 1996.

Post RM, Leverich GS, Altshuler LL, Frye MA, Suppes TM, Keck PE Jr, McElroy SL, Kupka R, Nolen WA, Grunze H, Walden J. An overview of recent findings of the Stanley Foundation Bipolar Network (Part I). Bipolar Disord 2003;5:310-319.

Pouwels PJ, Frahm J. Regional metabolites concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med 1998;39:53-60.

Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672–679.

Redpath TW, Smith FW. Technical note: Use of a double inversion recovery pulse sequence to image selectively grey or white brain matter. Br J Radiol 1994;67:1258–1263.

Rolls ET. The Brain and Emotion. Oxford University Press: Oxford, UK. 1999.

Sax KW, Strakowski SM, Zimmerman ME, DelBello MP, Keck Jr, PE, Hawkins JM. Frontosubcortical neuroanatomy and the continuous performance test in mania. Am J Psychiatry 1999;156:139-141.

Sharma R, Venkatasubramanian PN, Barany M, Davis JM. Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizophr Res 1992;8:43-49.

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE. Reduced frontal cortex inositol levels in post-mortem brain of suicide victims and patients with bipolar disorder. Am J Psychiatry 1997;154:1148-1150.

Silverstone PH, McGrath BM, Wessels PH, Bell EC, Ulrich M. Current pathophysiological findings in bipolar disorder and in its subtypes. Curr Psychiatry Rev 2005;1:75-101.

Silverstone PH, Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC. Chronic treatment with both lithium and sodium valproate may normalize phosphoinositol cycle activity in bipolar patients. Hum Psychopharmacol Clin Exp 2002;17:321-327.

Simpson SG, McMahon FJ, McInnis MG, MacKinnon DF, Edwin D, Folstein SE, DePaulo JR. Diagnostic reliability of bipolar II disorder. Arch Gen Psychiatry 2002;59:736-740.

Spaner D, Bland RC, Newman SC. Major Depressive Disorder. Acta Psychiatr Scand 1994;Suppl 376:7-15.

Stanley JA, Pettegrew JW, Keshavan MS. Magnetic resonance spectroscopy in schizophrenia: Methodological issues and findings – part I. Biol Psychiatry 2000;48:357-368.

Steele JD, Lawrie SM. Segregation of cognitive and emotional function in the prefrontal cortex: A stereotactic meta-analysis. Neuroimage 2004;21:868-875.

Stork C, Renshaw PF. Mitorchondrial dysfunction in bipolar disorder: Evidence from magnetic resonance spectroscopy research. Mol Psychiatry 2005;10:900-919.

Tohen M, Vieta E, Calabrese J, Ketter TA, Sachs G, Bowden C, Mitchell PB, Centorrino F, Risser R, Baker RW, Evans AR, Beymer K, Dube S, Tollefson GD, Breier A. Efficacy of olanzapine and olanzapine-fluoxetine combination in the treatment of bipolar I depression. Arch Gen Psychiatry 2003;60:1079-1088.

Tsai G, Coyle JT. N-Acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531-540.

Vogt BA, Finch DM, Olson CR. Functional heterogeneity in cingulated cortex: The anterior executive and posterior evaluative regions. Cereb Cortex 1992;2:435-443.

Weissman MM, Leaf PJ, Tischler GL, Blazer DG, Karno M, Bruce ML, Florio LP. Affective disorders in five United States communities. Psychol Med 1988;18:141-153.

Yatham LN, Kennedy SH, O'Donovan C, Parikh S, MacQueen G, McIntyre R, Sharma V, Silverstone P, Alda M, Baruch P, Beaulieu S, Daigneault A, Milev R, Young T, Ravindran A, Schaffer A, Connelly M, Gorman CP. Canadian network for mood and anxiety treatments (CANMAT) guidelines for the management of patients with bipolar disorder: Consensus and controversies. Bipolar Disord 2005;7(Suppl 3):5-69.

Yatham L, Kusumakar V, Parikh S, Haslam DR, Matte R, Sharma V, Kennedy S. Bipolar depression: Treatment options. Can J Psychiatry 1997;42(Suppl 2):87S-91S.

Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: Reliability, validity, and sensitivity. Br J Psychiatry 1978;133:429-435.

Zhong K, Ernst T. Localized in vivo human ^{1}H MRS at very short echo times. Magn Reson Med 2004;52:898-901.

Chapter 7: General Discussion and Conclusions

7.1 Overall Summary

Mood disorders, including BPD and UPD, are among the most prevalent and debilitating of the psychiatric illnesses. Moreover, they are among the leading causes of morbidity worldwide, affecting anywhere between 1% to 25% of people at some point during their lifetime. Of the symptoms experienced, depression is the most common and life threatening, particularly due to the increased risk of suicide during these episodes. Given both their prevalence and severity, it is remarkable that the most effective and commonly prescribed medication for BPD is the element lithium, while treatments for UPD have been steadily evolving over a number of decades. Recent advances have seen the application of several medications, from several different classes, including anticonvulsants and antipsychotics, to the treatment of BPD. And while this has meant better symptom relieve for patients, it has also complicated matters with respect to the understanding of illness pathophysiology.

The introduction of clinical nuclear magnetic resonance in the 1980's ushered in a new era in the study of brain physiology. For the first time, brain structure, function and chemistry could be studied *in vivo* non-invasively. With respect to brain chemistry, the application of MRS has meant that changes in specific metabolite concentrations can be measured during different symptom states and following successful treatment and recovery. In BPD, much of this research has focused on measuring brain *myo*-inositol concentrations, as a result of early preclinical work pointing to effects of lithium on this neurometabolite – the inositol depletion hypothesis of lithium action. *Myo*-inositol is the common breakdown product of the phosphatidylinositol second messenger system (PI-cycle). It is also possible to utilize the same basic methodology to study drug effects in animals *in vitro*. Much of the early MRS research in BPD was limited to relatively large brain volumes, employing metabolite non-specific data acquisition parameters in diagnostically mixed groups of patients. For instance, most MRS studies have not distinguished between patients with BPD-I and BPD-II, and no studies have compared either of the subtypes with UPD. Moreover, there is a dearth of information on the effects of medications other than lithium on *myo*-inositol concentration.

The series of experiments that form this doctoral thesis were designed to assess alterations in *myo*-inositol concentrations using MRS both *in vivo* and in animals *in vitro*. Differences in *myo*-inositol concentrations were evaluated in depressed BPD-II patients relative depressed UPD patients and HC subjects. This is the first study to look specifically at BPD-II patients in relation to UPD patients. This is of interest given that BPD-II spend much more of their time depressed than they do hypomanic and given that this subtype of BPD is far more common then is BPD-I. The effects of acute dextro-amphetamine, a putative model for mania, on *myo*-inositol concentration in HV and in rats was also evaluated. In humans, dextro-amphetamine produces subjective and behavioural symptoms similar to those reported by patients with mania or hypomania, including increased blood pressure, nervousness, and loss of appetite among others. As such, it would be of great interest to determine if these changes are mirrored by neurochemical changes that have also been reported in patients with mania or hypomania. The effect of lithium treatment length on regional rat brain *myo*-inositol concentrations was also assessed to determine whether lithium-induced reductions in *myo*-inositol are tied to those brain regions implicated in the clinical manifestation of mood disorders. Finally, the effects on regional rat brain *myo*-inositol concentrations of other commonly prescribed mood-stabilizers as well as antidepressants was also assessed. Lithium has been shown to reduce whole brain *myo*-inositol concentration; however, there is little evidence of its effects, or those of other mood stabilizers, on different brain regions. This would be of great interest and may enable the linking of treatment effects with neurochemical changes in particular anatomical structures.

The main results are: [1] *Myo*-inositol concentrations did not differ significantly between depressed BPD-II patients, depressed UPD patients and HC, although there was a numeric increase in the BPD-II group. Similarly, *myo*-inositol concentrations in depressed UPD patients did not differ significantly from HC values, with the UPD group having concentrations about midway between those of the BPD-II group and the HC. Thus, it appears that there are not alterations in *myo*-inositol concentrations between the two patient groups and between patients and HC. [2] An acute dose of dextro-amphetamine did not to alter *myo*-inositol concentrations in the brains of HV in a manner consistent with clinical changes observed in patients with bipolar mania. Similar results were found in four brain regions assessed in rats given acute dextro-amphetamine. Previous studies have reported increased *myo*-inositol concentrations in the brains of manic and hypomanic BPD patients; however, it is unlikely that dextro-amphetamine induces similar effects. [3] Long-term (2 and 4 week) but not short-term (1 week) treatment with lithium reduces regional rat brain (prefrontal, temporal occipital cortices as well as hippocampus) *myo*-inositol concentrations in a manner consistent with the inositol depletion hypothesis. Interestingly, treatment with lithium reduced *myo*-inositol concentrations in all four brain regions, a global effect. The clinical literature has not reported *myo*-inositol alterations in the occipital cortex of BPD patients who are symptomatic or who have been successfully treated with lithium. It appears that lithium's effect on the PI-cycle may be more global, which makes it difficult to determine its particular relevance to the treatment of mood disorders. [4] Contrary to the effects of lithium, the mood stabilizers lamotrigine, sodium valproate and carbamazepine do not affect regional *myo*-inositol concentrations in the brains of rats. [5] Similarly, antidepressants belonging to three distinct chemical classes (i.e. SSRIs, TCAs and MAOIs) do not change regional *myo*-inositol concentrations in the brains of rats. Thus, a change in *myo*-inositol concentrations appears to be an effect unique to lithium, and is not likely a mechanism involved in the treatment effectiveness of the other mood stabilizers and the antidepressants evaluated in the present series of studies.

7.2 Limitations

All the results of the present series of experiments must be appraised with the limitations of said experiments in mind. With respect to the study of the acute effects of dextro-amphetamine, the primary limitation was that this study assessed the effects of a single dose of dextro-amphetamine. It is possible that while a single dose of dextro-amphetamine did not alter *myo*-inositol concentrations, chronic treatment would. This would be analogous to the findings in the lithium experiment, where one-week treatment had no effect, but longer treatments reduced *myo*-inositol concentrations. Indeed, there is evidence that chronic use of methamphetamine increases *myo*-inositol concentrations (Chang et al. 2005). In a study of human immunodeficiency virus (HIV) and methamphetamine abuse, HIV-negative methamphetamine abusers had significantly higher frontal grey matter, but not white matter, *myo*-inositol concentrations compared non-methamphetamine abusers with or without HIV (Chang et al. 2005). Differences in *myo*-inositol concentrations did not correlate with the frequency of methamphetamine use, the dose administered, duration of use or the cumulative dose administered. It is difficult to determine whether the changes in *myo*-inositol concentrations are a direct result of chronic exposure to methamphetamine or an effect secondary to other methamphetamine-induced pathologies, such as neuronal death and increase in astroglia density. This is possible. From an ethical perspective, due to the risk of dependence it would not be advisable to treat HV chronically with a CNS stimulant like amphetamine, even at low or modest doses. There are no long-term studies of the effects of dextro- or methamphetamine on non-human brain *myo*-inositol concentrations. Acute dextro-amphetamine was found to induce subjective and physiological changes consistent with similar measures in manic/hypomanic BPD patients and consistent with findings in chronic amphetamine abusers. Thus, in so far as dextro-amphetamine models mania or hypomania, it is unlikely that chronic treatment would have induced *myo*-inositol concentration changes consistent with this disorder.

In the study of depressed BPD-II and UPD patients, the main limitations are the small number of BPD-II patients included in the study and the fact that not all patients were medication-free at the time of the scan. These limitations highlight the difficulty in recruiting patients to participate and in finding patients that have not been treated (or are not currently being treated) but are actually ill enough to participate in the study. Magnetic resonance spectroscopy studies in euthymic BPD patients have not shown differences in *myo*-inositol concentrations relative to HC subjects, independent of whether or not the patients were receiving treatment. It could be argued that euthymic or asymptomatic patients have *myo*-inositol concentrations that fall within the healthy (or normal) range and that drug treatment works only to correct pathology and not to alter an already healthy environment. This is supported by MRS investigations of euthymic BPD patients (Bruhn et al. 1993; Winsberg et al. 2000; Chang et al. 2003) and also by findings in HV given lithium or sodium valproate, with no observable effect on *myo*-inositol concentration (Silverstone 1996, 1999; Brambilla et al. 2004). In this way, it could also be argued that symptomatic patients who are treated could be expected to have similar underlying *myo*-inositol concentrations to those of untreated symptomatic patients, by virtue of the proposed relationship between symptom manifestation and changes in *myo*-inositol concentration. However, based on the results of the present study, it appears that there is no such relationship and that any associations between *myo*-inositol concentrations and mood disorder pathophysiology may only be secondary to some other pathologically-induced change(s) in brain physiology. This remains to be clarified.

Finally, a limitation that plagues all MRS investigations of brain chemistry is the fact that it is not yet possible to determine the functional significance, if any, of changes in metabolite concentrations for any region investigated. Given the highly evolved nature of the brain, particularly in mammalian species (Karten 1997), most neurochemicals, including neurotransmitters, are known to play numerous and diverse roles in neurons and surrounding glia, and may even be stored in a functionally specific manner. This may be true for *myo*-inositol as well, which is known to be involved in the PI-cycle, but is also involved in osmotic regulation and is a purported glial cell marker (Strange et al. 1991; Strange et al. 1992). Current MRS technology does not allow for a distinction of which function, or functions, are being affected by disease or impacted by treatment, although a better determination of one may provide significant insight into the other. Marrying new insights gained from preclinical experimentation with findings from the clinical milieu will likely provide greater insight into this issue.

7.3 Overall Conclusions and Future Directions

While changes in *myo*-inositol concentration have been linked to lithium treatment in humans and in rats, it appears that this effect may be unique to lithium. Other medications may act on the PI-cycle, but not through inositol depletion. For instance, while lithium may act by decreasing global *myo*-inositol concentrations, other medications may act by reducing the binding affinity of other PI-cycle enzymes, the resultant effect of both being to slow PI turnover and any dependent downstream events. This requires greater clarification. While amphetamine-induced subjective and physiological effects seem to mimic the symptoms of mania and hypomania, it is unlikely that these behavioural similarities share a common physiological basis. Finally, although changes in *myo*-inositol concentrations have been reported to be altered in BPD and to a lesser extent in UPD, the present study did not find any differences between depressed BPD-II patients, depressed UPD patients and HC, even after implementing important methodological improvements in detection, acquisition and quantification. It is thus unlikely that alterations in *myo*-inositol concentration are a marker of mood disorders in particular.

With technological advancements in MRS, future investigations should investigate the clinical and functional significance of altered neurometabolite concentrations. For instance, acquiring both MRS and fMRI or PET data would enable not only the measurement of regional *myo*-inositol concentrations, but also allow for the assessment of task-dependent functional changes or enzymatic activity and receptor binding. Those data, taken together, would not only provide greater insight into psychiatric disorders, like BPD and UPD, but would also open a window on the workings of the healthy human brain.

7.4 Bibliography

Brambilla P, Stanley JA, Sassi RB, Nicoletti MA, Mallinger AG, Keshavan MS, Soares JC. ^{1}H MRS study of dorsolateral prefrontal cortex in healthy individuals before and after lithium administration. Neuropsychopharmacol 2004;29:1918-1924.

Bruhn H, Stoppe G, Staedt J, Merboldt KD, Hanicke W, Frahm J. Quantitative proton MRS in vivo shows cerebral myo-inositol and cholines to be unchanged in manic-depressive patients treated with lithium. Proceedings of the Society of Magnetic Resonance in Medicine, August 14-20, 1993, New York. 1543.

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children and familial bipolar disorder. Biol Psychiatry 2003;53:1059-1065.

Chang L, Ernst T, Speck O, Grob CS. Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. Am J Psychiatry 2005;162:361-369.

Karten HJ. Evolutionary developmental biology meets the brain: The origins of mammalian cortex. Proc Natl Acad Sci USA 1997;94:2800-2804.

Silverstone PH, Rotzinger S, Pukhovsky A, Hanstock CC. Effects of lithium and amphetamine on inositol metabolism in the human brain as measured by 1H and 31P MRS. Biol Psychiatry 1999;46:1634-1641.

Silverstone PH, Hanstock CC, Fabian J, Staab R, Allen PS. Chronic lithium does not alter human myo-inositol or phosphomonoester concentrations as measured by 1H and 31P MRS. Biol Psychiatry 1996;40:235-246.

Strange K, Morrison R. Volume regulation during recovery from chronic hypertonicity in brain glial cells. Am J Physiol 1992;263:C412-C419.

Strange K, Morrison R, Heilig CW, DiPietro S, Gullans SR. Upregulation of inositol transport mediates inositol accumulation in hyperosmolar brain cells. Am J Physiol 1991;260:C784-C790.

Winsberg ME, Sachs N, Tate DL, Adalsteinsson E, Spielman D, Ketter TA. Decreased dorsolateral prefrontal N-acetylaspartate in bipolar disorder. Biol Psychiatry 2000;47:475-481.

Appendix A: Neurochemical Characterization of Rat Brain at 18.8 Tesla

A.1 Introduction

Unlike *in vivo* spectroscopy, *ex vivo* or high field NMR spectroscopy is able to detect numerous metabolites, from several tissue types, including brain. Spectroscopy conducted at lower field strengths typical of most clinical investigations, make it particularly difficult to measure metabolites acquired at short echo times. This generally results from the presence of several unresolved multiplets with complex line shapes, signals from lipids and other macromolecules, unsuppressed water, and the considerable spectral overlap between metabolites (e.g. *myo*-inositol and glycine). These difficulties can largely be overcome at higher field strengths, enabling the accurate detection and quantification of numerous metabolites. To follow is a brief overview of the metabolites that can be readily identified at 18.8T from several regions of the rat brain and the effects of several classes of psychiatric medication on their concentrations.

A.2 Materials and Methods

Data presented herein were obtained during the experiments examining *myo*-inositol, and employ the same methodology. Briefly, all rats were treated with medications used in the treatment of BPD and UPD, at doses previously shown to produce therapeutically relevant concentrations and/or to induce physiological responses related to those being assessed (Ghoshdastidar et al 1989; O'Donnell et al 2000; Del Arco et al 1998; Anderzhanova et al 2001; Mcgeehan et al 2004; Choe et al 2002; Andiarzhanova et al 2002; Anderzhanova et al 2002; Castel-Branco et al 2003; Hasegawa et al 2003; Chen et al 1999; Dwivedi et al. 2002). After treatment, rats were decapitated and the brains were rapidly removed and immediately frozen by immersing in 2-methylbutane kept on dry ice. At the time of dissection, whole brains were dissected into pre-frontal, temporal and occipital cortex, as well as hippocampus according to stereotaxic demarcation. Metabolites were retrieved using a well-established methanol/chloroform extraction procedure. At the time of NMR analysis, dried samples were reconstituted in 0.6 ml of distilled water and 0.06 ml of D_2O containing 5 mM DSS as an internal reference standard at a pH of approximately 7. DSS also serves as the chemical shift reference (set to 0 ppm). Spectroscopic data were acquired at 37°C on a Varian Inova-800 spectrometer (18.8T) equipped with a 5 mm triple axis gradient HCN probe. One-dimensional single 90° pulse ^1H spectra were collected with a water pre-saturation period of 2 seconds (γB_1 of ~ 150 Hz), sweep widths of 12000 Hz, and acquisition times of 2 seconds. All directly and indirectly detected data sets were zero filled to twice the number of acquired points. The 1D-^1H spectra were apodized using a 0.5 Hz line broadening or a cosine weighting function, respectively. Identification and quantification of metabolites from brain extracts were done using Chenomx Profiler NMRsuite software, which contains a database of metabolite molecules whose unique NMR spectral signatures are encoded at various spectrometer frequencies including 18.8 T. Comparison of the rat brain extract NMR spectra to the Chenomx spectral signature database within Profiler results in a list of compounds together with their respective concentrations, quantified by reference to the known amount of internal standard (DSS). All compounds in the database, including those discussed in this work, have been verified against known concentrations of reference NMR spectra of the pure compounds, enabling accurate metabolite identification and concentration quantification. Treatment effects were analyzed using either the Independent samples t-test or a one-way ANOVA, depending on experimental conditions, with significance evaluated at the $\alpha=0.05$ level, using SPSS© (version 11.0.4 for OS X 10.4.2). This analysis is exploratory in nature and is intended to serve only as a reference and to guide future, *a priori*, investigations of these metabolites. Statistical analysis was not corrected for multiple comparisons.

A.3 Results

Table A1 presents the rat brain metabolite concentrations of the pre-frontal cortex, temporal cortex, occipital cortex and hippocampus that are both detectable and identifiable with ^{1}H MRS at 18.8 Tesla. These data are from the vehicle-treated animals. Also, where available in the literature, data on the concentration ranges of these metabolites in the adult human brain are reported. To follow is a discussion of each metabolite in turn, including the effects of treatment on regional rat brain concentration.

Table A1: Rat Brain Metabolite Concentrations using ^{1}H MRS at 18.8 Tesla.

Compound	Rat Brain Concentrations (µmol/g wet weight)*				Normal Adult Human Brain Concentrations** (µmol/g wet weight)
	Pre-frontal Cortex	Hippocampus	Occipital Cortex	Temporal Cortex	
Glutamate	2.40 ± 0.16	4.57 ± 0.28	6.88 ± 0.39	6.22 ± 0.35	6.00-12.50
Lactate	1.89 ± 0.13	4.41 ± 0.31	5.28 ± 0.42	5.83 ± 0.99	0.40
Creatine	1.85 ± 0.14	4.54 ± 0.33	5.91 ± 0.50	5.71 ± 1.02	5.10-10.60
N-Acetylasparate	1.56 ± 0.12	2.97 ± 0.19	4.36 ± 0.34	4.94 ± 0.77	7.90-16.60
Glutamine	1.35 ± 0.10	2.69 ± 0.18	3.72 ± 0.31	3.87 ± 0.68	3.00-5.80
Aspartate	0.92 ± 0.08	1.67 ± 0.08	2.52 ± 0.16	3.00 ± 0.16	1.00-1.40
Taurine	1.13 ± 0.08	2.24 ± 0.13	2.69 ± 0.14	2.83 ± 0.16	0.90-1.50
γ-Aminobutyric Acid	0.73 ± 0.05	1.50 ± 0.10	1.79 ± 0.15	2.64 ± 0.53	1.30-1.90
Hypoxanthine	0.26 ± 0.03	0.59 ± 0.06	0.68 ± 0.06	0.81 ± 0.12	<0.10
Adenosine	0.20 ± 0.02	0.74 ± 0.06	0.85 ± 0.07	0.89 ± 0.18	<0.10
Serine	0.28 ± 0.03	0.54 ± 0.04	0.64 ± 0.05	0.78 ± 0.17	0.40
Glycine	0.22 ± 0.02	0.48 ± 0.03	0.62 ± 0.04	0.68 ± 0.13	0.40-1.00
Alanine	0.22 ± 0.02	0.46 ± 0.03	0.52 ± 0.05	0.62 ± 0.13	0.20-1.40
Phosphorylcholine	0.30 ± 0.02	0.43 ± 0.03	0.59 ± 0.05	0.68 ± 0.16	0.90-2.50
Choline	0.13 ± 0.01	0.32 ± 0.02	0.40 ± 0.03	0.41 ± 0.08	

Note: Concentrations reported as mean ± SEM. *Values represent the average concentrations of all vehicle treated rats, averaged across all treatment studies. **Values obtained from the following sources: Kovacs et al. 2005; van Zijl et al. 1993; Petroff et al. 1989; Kreis 1997; Klunk et al. 1996; Tan et al. 1998; Michaelis et al. 1993; van Zijl and Barker 1997; Siegel et al. 1989.

Table A1: Rat Brain Metabolite Concentrations Detected and Identified using ^{1}H MRS at 18.8 Tesla (continued).

Compound	Rat Brain Concentrations (µmol/g wet weight)*				Normal Adult Human Brain Concentrations** (µmol/g wet weight)
	Pre-frontal Cortex	Hippocampus	Occipital Cortex	Temporal Cortex	
Threonine	0.23 ± 0.02	0.26 ± 0.02	0.29 ± 0.02	0.47 ± 0.08	0.30
Acetate	0.11 ± 0.01	0.20 ± 0.01	0.30 ± 0.02	0.34 ± 0.08	0.40-0.80
Leucine	0.11 ± 0.01	0.18 ± 0.01	0.26 ± 0.03	0.29 ± 0.08	
Niacinamide	<0.10	0.16 ± 0.01	0.18 ± 0.02	0.22 ± 0.04	
Formate	<0.10	<0.10	<0.10	0.11 ± 0.02	
Valine	<0.10	<0.10	0.11 ± 0.01	0.13 ± 0.03	0.10
Succinate	<0.10	<0.10	<0.10	<0.10	0.40
Fumarate	<0.10	<0.10	<0.10	<0.10	
Uracil	<0.10	<0.10	<0.10	0.10 ± 0.03	<0.10
Histidine	<0.10	<0.10	<0.10	0.23 ± 0.11	<0.10
Isoleucine	<0.10	<0.10	0.11 ± 0.01	0.22 ± 0.07	

Note: Concentrations reported as mean ± SEM. *Values represent the average concentrations of all vehicle treated rats, averaged across all treatment studies. **Values obtained from the following sources: Kovacs et al. 2005; van Zijl et al. 1993; Petroff et al. 1989; Kreis 1997; Klunk et al. 1996; Tan et al. 1998; Michaelis et al. 1993; van Zijl and Barker 1997; Siegel et al. 1989.

Glutamate

In brain, glutamate is the most abundant excitatory amino acid. The majority of glutamate molecules are synthesized from glucose, via transamination of a α-ketoglutarate intermediate formed during the citric acid cycle in both neurons and glia (Peng et al. 1993). Currently, four major glutamate receptor subtypes are known: N-methyl-D-asparate, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, kainite receptors, and G-protein coupled metabotropic receptors (Choi et al. 2005). Altered glutamate neurotransmission has been suggested to be involved in mood disorders (Kugaya and Sanacora 2005) and schizophrenia (Nanitsos et al. 2005). Recent evidence also suggests that thalamic glutamate may be elevated in patients with generalized epilepsy (Helms et al. 2006). Glutamate is also detectable at higher clinical field strengths. In the present set of experiments (Table A2), treatment with acute dextro-amphetamine was found to significantly increase temporal cortex glutamate concentrations relative to vehicle. There was a trend toward an increase across the remaining three brain regions, but the results were not statistically different. Across the four brain regions assessed, treatment with each of three antidepressants (desipramine, fluoxetine and phenelzine) or the three anticonvulsants (sodium valproate, carbamazepine and lamotrigine) did not alter glutamate concentrations significantly from those of vehicle. One week of lithium treatment did not affect glutamate levels. Treatment for 2 and 4 weeks significantly reduced glutamate levels across all 4 brain regions relative to vehicle. Lithium-induced reductions in glutamate concentrations have been reported previously in rat whole brain (O'Donnell et al. 2000; Plenge 1976) and substantia nigra (Jope et al. 1989), while increases have been reported in the brain stem (Marcus et al. 1986) and hypothalamus (Gottesfeld 1976), using different methodology.

Table A2: Glutamate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	5.57 ± 1.19	4.38 ± 0.42	11.14 ± 2.06*	7.83 ± 0.82
Vehicle	4.32 ± 1.20	3.32 ± 0.18	5.97 ± 0.62	6.00 ± 1.24
Desipramine	3.82 ± 0.84	3.75 ± 0.47	8.02 ± 1.94	6.57 ± 0.88
Fluoxetine	3.30 ± 0.60	4.09 ± 0.32	7.30 ± 1.61	8.59 ± 0.66
Phenelzine	2.46 ± 0.53	6.06 ± 0.88	6.33 ± 1.11	4.92 ± 1.08
Vehicle	2.10 ± 0.22	5.84 ± 0.82	6.11 ± 1.14	6.01 ± 0.92
Lamotrigine	2.92 ± 0.67	5.20 ± 1.35	5.10 ± 0.83	4.91 ± 0.74
Sodium Valproate	3.23 ± 0.70	6.56 ± 1.94	4.53 ± 0.68	4.44 ± 0.45
Carbamazepine	3.20 ± 0.28	3.52 ± 0.24	3.73 ± 0.37	3.87 ± 0.29
Vehicle	2.42 ± 0.40	5.22 ± 1.08	3.56 ± 0.42	5.37 ± 1.50
1 Week Tx				
LiCl	2.34 ± 0.39	3.78 ± 1.00	6.44 ± 0.43	6.85 ± 0.37
Vehicle	2.87 ± 0.40	3.84 ± 0.50	6.85 ± 0.98	7.92 ± 0.46
2 Week Tx				
LiCl	1.10 ± 0.08*	3.26 ± 0.23*	5.11 ± 0.25**	6.29 ± 0.45*
Vehicle	2.32 ± 0.44	4.36 ± 0.38	6.59 ± 0.24	7.92 ± 0.26
4 Week Tx				
LiCl	1.32 ± 0.14*	3.09 ± 0.22**	4.98 ± 0.43*	6.56 ± 0.27**
Vehicle	2.29 ± 0.30	4.86 ± 0.27	6.20 ± 0.37	8.07 ± 0.28

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05, **p<0.005

Lactate

Changes in lactate concentration are primarily produced during anaerobic respiration, of which lactate is the end product of glycolysis (Berg et al. 2002). Although it is constantly produced during normal metabolism, the concentration of lactate remains relatively low and stable unless the rate of production exceeds the rate of removal (Veech 1991). Pathologically, lactate concentration also increases following hypoxia, such as during strokes or tumors, and during hyperventilation (Rudkin and Arnold 1999). Lactate has also be used as a panicogen to induce anxiety in patients with panic disorder (Keck and Strohle 2005). In the present set of experiments (Table A3), treatment with acute dextro-amphetamine did not significantly change lactate concentration in the brain regions assessed, relative to vehicle. Similarly, treatment with antidepressants or anticonvulsants did not affect lactate concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a non-significant increase at 2 weeks and a significant increase at 4 weeks in the hippocampus. A significant increase in the occipital cortex was also observed at 2 weeks. A similar relationship was observed in the temporal cortex, but this was not significant.

Table A3: Lactate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	4.13 ± 0.70	4.44 ± 0.45	9.74 ± 2.00	7.61 ± 0.74
Vehicle	4.49 ± 1.03	3.64 ± 0.27	6.35 ± 0.89	6.56 ± 1.29
Desipramine	3.43 ± 0.75	4.39 ± 0.57	7.43 ± 1.52	5.72 ± 0.89
Fluoxetine	2.95 ± 0.49	4.33 ± 0.35	7.18 ± 2.16	7.00 ± 0.67
Phenelzine	2.45 ± 0.54	6.15 ± 0.84	6.26 ± 1.07	4.13 ± 0.95
Vehicle	1.78 ± 0.18	5.56 ± 0.91	6.89 ± 1.14	4.38 ± 0.65
Lamotrigine	2.62 ± 0.66	4.36 ± 0.98	3.76 ± 0.57	3.06 ± 0.33
Sodium Valproate	3.11 ± 0.66	5.10 ± 1.22	3.79 ± 0.50	3.45 ± 0.58
Carbamazepine	2.64 ± 0.34	3.29 ± 0.16	2.92 ± 0.22	2.79 ± 0.17
Vehicle	2.22 ± 0.38	4.38 ± 0.90	3.23 ± 0.24	3.83 ± 1.07
1 Week Tx				
LiCl	2.90 ± 0.39	3.56 ± 0.96	6.71 ± 1.27	4.63 ± 0.34
Vehicle	2.18 ± 0.25	4.26 ± 0.85	11.00 ± 5.56	7.19 ± 1.38
2 Week Tx				
LiCl	2.18 ± 0.20	5.84 ± 0.72	5.18 ± 0.84	7.33 ± 1.14*
Vehicle	1.55 ± 0.32	4.88 ± 1.05	3.75 ± 0.31	4.58 ± 0.46
4 Week Tx				
LiCl	2.36 ± 0.36	6.76 ± 1.06*	5.95 ± 1.73	4.36 ± 0.47
Vehicle	1.72 ± 0.23	3.76 ± 0.31	3.75 ± 0.26	5.14 ± 0.52

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Creatine

In the cell, creatine functions as an energy exchanger. Adenosine triphosphate (or ATP) is formed by the transfer of a high-energy phosphate group from phosphocreatine to adenosine diphosphate (ADP). Adenosine diphospate is regenerated by the transfer of this phosphate group from ATP to creatine, reforming phosphocreatine. This energy shuttle helps to maintain a stable ATP/ADP ratio within the cell (Berg et al. 2002). Creatine is thus an important marker of cellular energy metabolism and turnover. Creatine (+ phosphocreatine) is another metabolite that is NMR-visible at clinical field strengths. Moreover, this metabolite is often used as the internal concentration reference, serving as the denominator in ratio quantification approaches. This approach has been predicated on the notion that creatine concentration does not change with age (Saunders et al. 1999), treatment or in different medical conditions. This is now being called into question, and there is evidence in the bipolar disorder literature to suggest that creatine is not stable and unchanging (Hamakawa et al. 1999; Kato et al. 1995; Deicken et al. 2001). Interestingly, to date this metabolite has not received much direct investigation in the clinical MRS literature. In the present set of experiments (Table A4), treatment with acute dextro-amphetamine showed a strong trend toward an increase in creatine, but this did not reach statistical significance for any region. Clinically, studies have not reported differences in creatine levels among methamphetamine users (Nordahl et al. 2005). Treatment with antidepressants or anticonvulsants did not affect creatine concentration in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase at 4 weeks in the hippocampus. There were no other differences across brain regions and treatment lengths. In humans lithium was found not to alter creatine levels in the DLPFC (Brambilla et al. 2004). There have been no human MRS studies of lithium's action in the hippocampus.

Table A4: Creatine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	3.69 ± 0.82	3.99 ± 0.36	8.00 ± 1.74	5.94 ± 0.48
Vehicle	3.46 ± 0.86	3.10 ± 0.13	4.50 ± 0.46	4.92 ± 0.89
Desipramine	2.34 ± 0.52	3.50 ± 0.44	5.67 ± 1.19	4.84 ± 0.75
Fluoxetine	2.03 ± 0.37	3.41 ± 0.29	5.18 ± 1.42	5.64 ± 0.49
Phenelzine	1.64 ± 0.37	4.81 ± 0.66	4.69 ± 0.82	3.32 ± 0.78
Vehicle	1.26 ± 0.16	4.46 ± 0.65	5.47 ± 0.76	3.93 ± 0.62
Lamotrigine	1.86 ± 0.45	3.89 ± 0.83	3.28 ± 0.52	3.03 ± 0.33
Sodium Valproate	2.27 ± 0.40	4.68 ± 1.32	3.31 ± 0.47	3.00 ± 0.27
Carbamazepine	2.02 ± 0.20	2.91 ± 0.17	2.70 ± 0.26	2.84 ± 0.18
Vehicle	1.74 ± 0.32	4.12 ± 0.99	2.75 ± 0.22	3.72 ± 0.96
1 Week Tx				
LiCl	2.47 ± 0.39	4.21 ± 1.12	7.74 ± 1.58	5.72 ± 0.44
Vehicle	2.52 ± 0.30	5.00 ± 0.95	12.19 ± 5.60	9.51 ± 1.67
2 Week Tx				
LiCl	1.84 ± 0.17	5.95 ± 0.63	5.45 ± 0.90	8.76 ± 1.40
Vehicle	1.75 ± 0.36	5.58 ± 1.13	4.62 ± 0.34	6.28 ± 0.56
4 Week Tx				
LiCl	2.10 ± 0.19	8.48 ± 1.46*	6.42 ± 1.84	5.55 ± 0.53
Vehicle	1.97 ± 0.30	4.97 ± 0.50	4.72 ± 0.42	7.07 ± 0.72

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

N-Acetylaspartate

N-Acetylaspartate is the most readily MRS-detectable neurometabolite at clinical field strengths, and as such comprises the largest peak, besides water, that is visible in standard ^{1}H-MRS. NAA is found predominantly in axons of neurons and is purported to be involved in synthetic processes within these structures, and thus may provide a marker of neuronal integrity (Malhi et al. 2002). Decreased NAA has been proposed to reflect neuronal degeneration (Tsai and Coyle 1995), making it an important target of neurodegenerative and psychiatric investigation. In the present set of experiments (Table A5), treatment with acute dextro-amphetamine significantly increased NAA concentration in the hippocampus, but not in other regions, relative to vehicle. Interestingly, in rats with neonatal hippocampal damage, NAA levels are reduced (Bertolino et al. 2002). This increase in NAA may be temporary, as long-term methamphetamine users have been reported to have reduced levels in several brain regions (Nordahl et al. 2005; Nordahl et al. 2002). Treatment with antidepressants or anticonvulsants did not affect NAA concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase in NAA at 4 weeks in the hippocampus. There were no other differences across brain regions and treatment lengths. Patients with bipolar disorder have been reported to have lower than control levels of hippocampal NAA (Bertolino et al. 2003). Lithium may act to normalize these levels.

Table A5: *N*-Acetylaspartate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	3.49 ± 0.80	2.88 ± 0.24*	7.34 ± 1.65	5.01 ± 0.50
Vehicle	3.21 ± 0.74	2.22 ± 0.13	4.16 ± 0.54	4.32 ± 0.82
Desipramine	1.85 ± 0.38	2.38 ± 0.26	5.17 ± 1.06	3.04 ± 0.53
Fluoxetine	1.66 ± 0.29	2.44 ± 0.17	4.67 ± 1.28	4.27 ± 0.39
Phenelzine	1.44 ± 0.29	3.37 ± 0.46	4.48 ± 0.67	2.48 ± 0.56
Vehicle	1.16 ± 0.13	3.43 ± 0.46	4.94 ± 0.67	3.14 ± 0.44
Lamotrigine	1.87 ± 0.44	2.90 ± 0.66	3.17 ± 0.41	2.50 ± 0.32
Sodium Valproate	2.04 ± 0.47	3.39 ± 0.95	2.98 ± 0.35	2.35 ± 0.17
Carbamazepine	1.99 ± 0.25	2.06 ± 0.11	2.59 ± 0.26	2.21 ± 0.12
Vehicle	1.74 ± 0.35	2.76 ± 0.54	2.63 ± 0.26	2.82 ± 0.71
1 Week Tx				
LiCl	2.13 ± 0.34	2.70 ± 0.78	6.11 ± 1.26	4.05 ± 0.32
Vehicle	1.97 ± 0.27	3.17 ± 0.61	9.80 ± 4.24	6.53 ± 1.14
2 Week Tx				
LiCl	1.45 ± 0.11	3.85 ± 0.46	4.54 ± 0.78	5.86 ± 0.89
Vehicle	1.44 ± 0.28	3.34 ± 0.65	4.08 ± 0.35	4.53 ± 0.44
4 Week Tx				
LiCl	1.74 ± 0.20	5.29 ± 0.91*	5.83 ± 1.63	4.02 ± 0.93
Vehicle	1.52 ± 0.22	2.90 ± 0.25	4.00 ± 0.28	4.84 ± 0.51

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Glutamine

During neurotransmission, unbound presynaptically-released extracellular glutamate is taken up by glial astrocytes, where it is converted to glutamine. Glutamine is then transported back to the nerve terminal where it is converted back into glutamate in the mitochondria. This process has been referred to as the glutamate–glutamine cycle (Nicklas et al. 1987). Importantly, the metabolic interconversion of glutamate–glutamine–glutamate is the only known process through which glutamine can be synthesized. Moreover, as glutamine is a synaptically-inactive compound it is thought that its function in brain is to serve as a storage reservoir for glutamate (Feldman et al. 1997). At clinical field strengths, glutamine cannot be measured directly, but is a minor contributor to the peak assigned to glutamate. In the present set of experiments (Table A6), treatment with acute dextro-amphetamine did not significantly change glutamine concentrations in the brain regions assessed, relative to vehicle. Similarly, treatment with antidepressants or anticonvulsants did not affect glutamine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase in glutamine at 4 weeks in the hippocampus. There were no other differences across brain regions and treatment lengths.

Table A6: Glutamine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	2.29 ± 0.36	2.22 ± 0.15	5.45 ± 1.09	3.53 ± 0.34
Vehicle	2.67 ± 0.72	1.94 ± 0.06	3.81 ± 0.52	3.39 ± 0.61
Desipramine	1.93 ± 0.43	2.01 ± 0.22	4.35 ± 0.95	3.49 ± 0.51
Fluoxetine	1.72 ± 0.32	2.18 ± 0.15	4.24 ± 1.18	4.25 ± 0.38
Phenelzine	1.11 ± 0.26	2.52 ± 0.30	3.20 ± 0.56	1.98 ± 0.43
Vehicle	1.09 ± 0.14	2.97 ± 0.36	4.66 ± 0.66	2.97 ± 0.40
Lamotrigine	1.71 ± 0.48	2.74 ± 0.89	2.54 ± 0.41	2.37 ± 0.42
Sodium Valproate	1.64 ± 0.29	2.96 ± 0.90	2.03 ± 0.21	2.10 ± 0.26
Carbamazepine	2.02 ± 0.56	2.19 ± 0.35	2.19 ± 0.30	2.54 ± 0.52
Vehicle	1.47 ± 0.28	2.62 ± 0.54	1.82 ± 0.15	2.62 ± 0.76
1 Week Tx				
LiCl	1.75 ± 0.24	2.29 ± 0.59	4.84 ± 0.79	3.15 ± 0.19
Vehicle	1.82 ± 0.22	2.68 ± 0.47	7.70 ± 3.77	5.58 ± 1.26
2 Week Tx				
LiCl	1.16 ± 0.10	3.00 ± 0.32	3.40 ± 0.90	4.79 ± 0.78
Vehicle	1.04 ± 0.18	3.22 ± 0.61	2.51 ± 0.19	3.59 ± 0.32
4 Week Tx				
LiCl	1.50 ± 0.16	4.80 ± 0.81*	3.72 ± 0.99	3.21 ± 0.29
Vehicle	1.31 ± 0.20	2.70 ± 0.32	2.71 ± 0.18	4.15 ± 0.42

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Aspartate

After glutamate, aspartate is the second most common excitatory amino acid found in neurons. It is purported to act as a neurotransmitter (Yuzaki et al. 1996), but its exact role is not clear. In the present set of experiments (Table A7), treatment with acute dextro-amphetamine did not significantly change aspartate concentration in the brain regions assessed, relative to vehicle. Treatment with fluoxetine significantly increased asparate concentrations in the occipital cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect aspartate concentration in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant decrease in aspartate concentrations at 2 weeks in the temporal cortex and at 4 weeks in both the temporal and occipital cortices. There was a trend toward a decrease across all conditions, but these did not reach significance.

Table A7: Aspartate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	2.20 ± 0.47	1.66 ± 0.14	4.27 ± 1.03	2.76 ± 0.17
Vehicle	2.57 ± 0.73	1.52 ± 0.10	2.95 ± 0.35	2.99 ± 0.59
Desipramine	1.17 ± 0.27	1.38 ± 0.17	2.93 ± 0.71	2.17 ± 0.33
Fluoxetine	1.13 ± 0.25	1.41 ± 0.16	2.83 ± 0.90	2.72 ± 0.22*
Phenelzine	0.79 ± 0.15	1.74 ± 0.22	2.35 ± 0.34	1.35 ± 0.25
Vehicle	0.74 ± 0.13	1.76 ± 0.23	3.19 ± 0.46	1.76 ± 0.23
Lamotrigine	0.90 ± 0.23	1.55 ± 0.24	1.82 ± 0.26	1.51 ± 0.12
Sodium Valproate	1.16 ± 0.18	1.92 ± 0.54	1.66 ± 0.19	1.36 ± 0.08
Carbamazepine	0.99 ± 0.11	1.14 ± 0.06	1.44 ± 0.15	1.31 ± 0.09
Vehicle	1.20 ± 0.25	1.62 ± 0.29	1.64 ± 0.10	1.84 ± 0.42
1 Week Tx				
LiCl	0.98 ± 0.14	1.35 ± 0.28	3.66 ± 0.18	2.52 ± 0.22
Vehicle	1.10 ± 0.10	1.53 ± 0.16	3.86 ± 0.41	3.02 ± 0.28
2 Week Tx				
LiCl	0.46 ± 0.06	1.53 ± 0.08	2.58 ± 0.11*	2.35 ± 0.15
Vehicle	0.77 ± 0.16	1.87 ± 0.24	3.22 ± 0.18	2.75 ± 0.10
4 Week Tx				
LiCl	0.53 ± 0.06	1.71 ± 0.10	2.39 ± 0.20*	2.27 ± 0.07*
Vehicle	0.80 ± 0.13	1.73 ± 0.07	3.13 ± 0.22	2.77 ± 0.16

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05

Taurine

Taurine has been called a conditionally-essential amino acid (Gaull 1986), which must be obtained from dietary sources by infants, but can be synthesized by children and adults (Gaull 1989; Laidlaw and Kopple 1987). Interestingly, taurine is also one of the only amino acids that is not metabolized or incorporated into proteins, but rather it remains unbound and free in the intracellular cytoplasm (Han et al. 2006). It has been reported to be involved in osmoregulation and the modulation of neurotransmitter action (Hardy and Norwood 1998). In the present set of experiments (Table A8), treatment with acute dextro-amphetamine significantly increased taurine concentrations in the hippocampus, relative to vehicle. There was a trend toward an increase in the remaining three regions, but these did not reach significance. Treatment with antidepressants or anticonvulsants did not affect taurine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant decrease in taurine concentrations at 2 weeks in the pre-frontal and occipital cortices and at 4 weeks across all regions. There was a trend toward a decrease across all conditions, but these did not reach significance.

Table A8: Taurine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	1.94 ± 0.37	2.23 ± 0.25*	4.48 ± 0.82	3.29 ± 0.28
Vehicle	1.90 ± 0.47	1.63 ± 0.07	2.75 ± 0.32	2.68 ± 0.45
Desipramine	1.57 ± 0.34	1.82 ± 0.25	2.93 ± 0.64	2.59 ± 0.41
Fluoxetine	1.38 ± 0.26	1.80 ± 0.16	2.72 ± 0.68	2.89 ± 0.25
Phenelzine	1.17 ± 0.25	2.58 ± 0.32	2.85 ± 0.45	1.94 ± 0.40
Vehicle	0.86 ± 0.10	2.38 ± 0.33	3.14 ± 0.46	2.03 ± 0.31
Lamotrigine	1.40 ± 0.33	2.34 ± 0.44	2.00 ± 0.30	1.91 ± 0.21
Sodium Valp.	1.42 ± 0.24	2.60 ± 0.83	1.81 ± 0.28	1.58 ± 0.13
Carbamazepine	1.31 ± 0.13	1.49 ± 0.10	1.40 ± 0.18	1.34 ± 0.28
Vehicle	1.23 ± 0.24	2.44 ± 0.55	1.66 ± 0.17	2.06 ± 0.47
1 Week Tx				
LiCl	1.22 ± 0.18	1.80 ± 0.36	3.27 ± 0.19	2.77 ± 0.14
Vehicle	1.39 ± 0.15	2.17 ± 0.30	3.28 ± 0.52	3.18 ± 0.16
2 Week Tx				
LiCl	0.53 ± 0.05*	1.75 ± 0.05	2.59 ± 0.22	2.48 ± 0.20*
Vehicle	1.03 ± 0.14	2.43 ± 0.28	2.95 ± 0.19	3.06 ± 0.12
4 Week Tx				
LiCl	0.50 ± 0.05**	1.88 ± 0.12*	2.59 ± 0.21*	2.46 ± 0.05**
Vehicle	1.13 ± 0.15	2.40 ± 0.11	3.19 ± 0.09	3.13 ± 0.15

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05, **p<0.005

Gamma-Aminobutyric Acid

Gamma-aminobutyric acid is the brain's primary inhibitory amino acid neurotransmitter, working antagonistically with glutamate at many nerve cell synapses. GABA concentrations have been shown to vary with age, gender and brain region (Chang et al. 2003). It is detectable at clinical field strengths as a minor component (like glutamine) of the glutamate peak. Reduced GABA brain concentrations have been associated with postpartum depression in women (Epperson et al. 2006), in patients with anxiety disorders (Goddard et al. 2001) and in patients with depression (Sanacora et al. 1999), while increased concentrations have been associated with citalopram treatment in healthy volunteers (Bhagwagar et al. 2004). In the present set of experiments (Table A9), treatment with acute dextro-amphetamine showed a strong trend toward an increase in GABA, but this did not reach statistical significance for any region. Treatment with antidepressants or anticonvulsants did not affect GABA concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a strong trend toward an increase in GABA concentration at 2 and 4 weeks in the pre-frontal cortex, temporal cortex and hippocampus, but these did not reach significance relative to vehicle. No trend existed in the occipital cortex.

Table A9: Gamma-aminobutyric acid Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	1.71 ± 0.40	1.63 ± 0.19	2.89 ± 0.61	2.05 ± 0.13
Vehicle	1.70 ± 0.40	1.28 ± 0.07	1.79 ± 0.28	1.72 ± 0.30
Desipramine	1.21 ± 0.28	1.42 ± 0.16	2.62 ± 0.58	1.89 ± 0.29
Fluoxetine	1.04 ± 0.19	1.33 ± 0.11	2.24 ± 0.64	1.98 ± 0.18
Phenelzine	0.76 ± 0.18	1.91 ± 0.26	2.07 ± 0.37	1.28 ± 0.30
Vehicle	0.55 ± 0.06	1.60 ± 0.27	2.11 ± 0.29	1.22 ± 0.18
Lamotrigine	0.90 ± 0.20	1.38 ± 0.29	1.36 ± 0.20	0.93 ± 0.12
Sodium Valproate	1.22 ± 0.20	1.70 ± 0.52	1.46 ± 0.20	0.97 ± 0.09
Carbamazepine	0.91 ± 0.07	1.08 ± 0.08	1.15 ± 0.12	0.98 ± 0.07
Vehicle	0.82 ± 0.15	1.53 ± 0.35	1.18 ± 0.14	1.25 ± 0.35
1 Week Tx				
LiCl	1.19 ± 0.20	1.29 ± 0.36	3.05 ± 0.70	1.65 ± 0.14
Vehicle	0.92 ± 0.11	1.42 ± 0.23	5.60 ± 2.97	2.75 ± 0.53
2 Week Tx				
LiCl	0.76 ± 0.08	1.98 ± 0.25	1.98 ± 0.40	2.62 ± 0.48
Vehicle	0.58 ± 010	1.62 ± 0.33	1.35 ± 0.09	1.67 ± 0.14
4 Week Tx				
LiCl	0.82 ± 0.14	2.46 ± 0.47	1.81 ± 0.46	1.51 ± 0.21
Vehicle	0.76 ± 0.12	1.52 ± 0.17	1.55 ± 0.11	2.11 ± 0.22

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Hypoxanthine

Hypoxanthine is a naturally occurring purine derivative. It is a constituent of nucleic acids in the form of the nucleoside inosine (Berg et al. 2002). It has not been well studied in terms of its role in brain pathophysiology, but it has been linked to cognitive deficits in relation to Lesch-Nyhan disease, where there is a deficiency in the enzyme that catalyzes the conversion of hypoxanthine to its nucleotide (Seegmiller et al. 1967). In the present set of experiments (Table A10), treatment with acute dextro-amphetamine did not significantly change hypoxanthine concentration in the brain regions assessed, relative to vehicle. Treatment with desipramine significantly increased hypoxanthine concentration in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect hypoxanthine concentration in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter hypoxanthine concentrations in the brain regions assessed, relative to vehicle.

Table A10: Hypoxanthine Concentrations (µmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	0.92 ± 0.18	0.85 ± 0.14	1.50 ± 0.30	1.20 ± 0.08
Vehicle	1.12 ± 0.20	0.72 ± 0.04	1.06 ± 0.17	1.13 ± 0.20
Desipramine	0.75 ± 0.16*	0.94 ± 0.11	1.66 ± 0.32	1.28 ± 0.19
Fluoxetine	0.61 ± 0.10	0.79 ± 0.07	1.39 ± 0.35	1.35 ± 0.12
Phenelzine	0.42 ± 0.08	1.04 ± 0.14	1.13 ± 0.18	0.69 ± 0.15
Vehicle	0.33 ± 0.04	0.92 ± 0.14	1.20 ± 0.17	0.80 ± 0.10
Lamotrigine	0.58 ± 0.14	0.88 ± 0.18	0.85 ± 0.13	0.58 ± 0.05
Sodium Valproate	0.76 ± 0.12	1.07 ± 0.32	0.90 ± 0.11	0.68 ± 0.06
Carbamazepine	0.57 ± 0.06	0.63 ± 0.05	0.71 ± 0.08	0.64 ± 0.04
Vehicle	0.51 ± 0.10	0.86 ± 0.20	0.71 ± 0.06	0.78 ± 0.20
1 Week Tx				
LiCl	0.21 ± 0.03	0.28 ± 0.08	0.66 ± 0.14	0.34 ± 0.05
Vehicle	0.19 ± 0.02	0.31 ± 0.05	1.27 ± 0.56	0.55 ± 0.09
2 Week Tx				
LiCl	0.14 ± 0.01	0.41 ± 0.05	0.37 ± 0.04	0.60 ± 0.11
Vehicle	0.11 ± 0.02	0.35 ± 0.07	0.28 ± 0.03	0.36 ± 0.02
4 Week Tx				
LiCl	0.15 ± 0.02	0.46 ± 0.09	0.32 ± 0.07	0.32 ± 0.06
Vehicle	0.14 ± 0.02	0.36 ± 0.04	0.33 ± 0.03	0.46 ± 0.04

Note: Concentrations are reported as µmol/g wet weight (mean ± SEM).

*$p<0.05$

Adenosine

Adenosine, in the form of adenosine triphosphate is a nucleotide and the primary source of free energy in the cell (Berg et al. 2002). There is little literature on the involvement of adenosine in psychiatric disorders. In the present set of experiments (Table A11), treatment with acute dextro-amphetamine showed a strong trend toward an increase in adenosine, but this did not reach statistical significance for any region. Treatment with antidepressants or anticonvulsants did not affect adenosine concentrations in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter adenosine concentrations in the brain regions assessed, relative to vehicle.

Table A11: Adenosine Concentrations (µmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	0.58 ± 0.13	0.62 ± 0.04	1.18 ± 0.27	0.93 ± 0.10
Vehicle	0.56 ± 0.13	0.55 ± 0.04	0.82 ± 0.10	0.88 ± 0.17
Desipramine	0.30 ± 0.07	0.60 ± 0.08	0.99 ± 0.21	0.73 ± 0.10
Fluoxetine	0.25 ± 0.04	0.65 ± 0.06	0.99 ± 0.31	0.89 ± 0.08
Phenelzine	0.20 ± 0.05	0.94 ± 0.11	0.99 ± 0.19	0.54 ± 0.11
Vehicle	0.16 ± 0.02	0.84 ± 0.12	0.97 ± 0.13	0.62 ± 0.09
Lamotrigine	0.24 ± 0.06	0.67 ± 0.16	0.54 ± 0.09	0.39 ± 0.04
Sodium Valproate	0.36 ± 0.06	0.89 ± 0.25	0.68 ± 0.08	0.52 ± 0.04
Carbamazepine	0.32 ± 0.04	0.54 ± 0.02	0.54 ± 0.06	0.50 ± 0.04
Vehicle	0.27 ± 0.05	0.77 ± 0.20	0.54 ± 0.06	0.64 ± 0.16
1 Week Tx				
LiCl	0.29 ± 0.05	0.69 ± 0.18	1.19 ± 0.25	0.74 ± 0.05
Vehicle	0.26 ± 0.03	0.76 ± 0.20	1.92 ± 1.02	1.26 ± 0.21
2 Week Tx				
LiCl	0.16 ± 0.02	0.88 ± 0.10	0.70 ± 0.14	1.12 ± 0.20
Vehicle	0.15 ± 0.03	0.75 ± 0.16	0.46 ± 0.05	0.70 ± 0.03
4 Week Tx				
LiCl	0.18 ± 0.03	1.40 ± 0.28	0.78 ± 0.17	0.72 ± 0.14
Vehicle	0.19 ± 0.03	0.76 ± 0.09	0.64 ± 0.06	1.00 ± 0.12

Note: Concentrations are reported as µmol/g wet weight (mean ± SEM).

Serine

Serine is a non-essential amino acid commonly found in animal proteins, where it often serves an important role in the catalytic function of said proteins (Berg et al. 2002). It can be synthesized from glycine. Serine deficiency disorders are associated with microcephaly, seizures and psychomotor retardation (de Koning 2006). In the present set of experiments (Table A12), treatment with acute dextro-amphetamine did not significantly change serine concentration in the brain regions assessed, relative to vehicle. Treatment with antidepressants or anticonvulsants did not affect serine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase in serine concentrations at 2 weeks and a significant decrease at 4 weeks in occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A12: Serine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.39 ± 0.08	0.45 ± 0.02	0.92 ± 0.22	0.62 ± 0.05
Vehicle	0.38 ± 0.07	0.44 ± 0.04	0.53 ± 0.06	0.47 ± 0.08
Desipramine	0.36 ± 0.08	0.41 ± 0.04	0.79 ± 0.19	0.60 ± 0.10
Fluoxetine	0.31 ± 0.06	0.40 ± 0.03	0.77 ± 0.21	0.65 ± 0.06
Phenelzine	0.24 ± 0.05	0.63 ± 0.09	0.74 ± 0.17	0.46 ± 0.08
Vehicle	0.19 ± 0.02	0.54 ± 0.06	0.75 ± 0.10	0.47 ± 0.06
Lamotrigine	0.28 ± 0.07	0.54 ± 0.09	0.47 ± 0.07	0.42 ± 0.04
Sodium Valproate	0.39 ± 0.06	0.78 ± 0.27	0.53 ± 0.09	0.42 ± 0.04
Carbamazepine	0.29 ± 0.01	0.37 ± 0.03	0.38 ± 0.05	0.37 ± 0.03
Vehicle	0.25 ± 0.06	0.62 ± 0.15	0.42 ± 0.04	0.44 ± 0.12
1 Week Tx				
LiCl	0.34 ± 0.06	0.48 ± 0.12	1.23 ± 0.24	0.75 ± 0.05
Vehicle	0.36 ± 0.07	0.54 ± 0.08	1.89 ± 0.95	1.09 ± 0.16
2 Week Tx				
LiCl	0.23 ± 0.04	0.63 ± 0.05	0.83 ± 0.22	1.04 ± 0.16*
Vehicle	0.30 ± 0.04	0.58 ± 0.10	0.45 ± 0.05	0.56 ± 0.04
4 Week Tx				
LiCl	0.37 ± 0.05	0.86 ± 0.15	0.76 ± 0.16	0.51 ± 0.06*
Vehicle	0.31 ± 0.08	0.53 ± 0.06	0.63 ± 0.06	0.80 ± 0.07

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05

Glycine

Glycine is the simplest non-essential amino acid. It is primarily an inhibitory neurotransmitter, with receptors located primarily in the brain stem and spinal cord (Betz et al. 1999). Glycine has been linked to schizophrenia, due to the fact that it is a required co-agonist for channel activation of the *N*-methyl-*D*-aspartate receptor by glutamate (Thomson 1989). Moreover, administration of glycine as an adjunct to antipsychotic treatment has been reported to improve the negative symptoms in patients with treatment-resistent schizophrenia (Heresco-Levy 2002). In clinical MRS, glycine co-resonates with myo-inositol, and is a minor contributor to the peak at ~3.6 ppm. In the present set of experiments (Table A13), treatment with acute dextro-amphetamine did not significantly change glycine concentrations in the brain regions assessed, relative to vehicle. Treatment with antidepressants or anticonvulsants did not affect glycine concentrations in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter glycine concentrations in the brain regions assessed, relative to vehicle.

Table A13: Glycine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	0.41 ± 0.09	0.48 ± 0.05	0.99 ± 0.18	0.76 ± 0.06
Vehicle	0.57 ± 0.14	0.43 ± 0.03	0.63 ± 0.07	0.76 ± 0.11
Desipramine	0.36 ± 0.09	0.45 ± 0.06	0.78 ± 0.18	0.65 ± 0.09
Fluoxetine	0.30 ± 0.06	0.43 ± 0.03	0.66 ± 0.17	0.73 ± 0.07
Phenelzine	0.20 ± 0.04	0.50 ± 0.06	0.57 ± 0.10	0.38 ± 0.08
Vehicle	0.16 ± 0.02	0.50 ± 0.06	0.66 ± 0.10	0.44 ± 0.07
Lamotrigine	0.29 ± 0.07	0.47 ± 0.10	0.42 ± 0.06	0.35 ± 0.03
Sodium Valproate	0.36 ± 0.05	0.58 ± 0.18	0.46 ± 0.07	0.37 ± 0.03
Carbamazepine	0.32 ± 0.04	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.04
Vehicle	0.25 ± 0.05	0.48 ± 0.12	0.35 ± 0.03	0.45 ± 0.12
1 Week Tx				
LiCl	0.35 ± 0.06	0.43 ± 0.11	1.00 ± 0.22	0.61 ± 0.08
Vehicle	0.28 ± 0.04	0.46 ± 0.08	1.56 ± 0.72	0.86 ± 0.15
2 Week Tx				
LiCl	0.22 ± 0.02	0.64 ± 0.07	0.59 ± 0.09	0.95 ± 0.16
Vehicle	0.18 ± 0.04	0.52 ± 0.10	0.43 ± 0.03	0.58 ± 0.04
4 Week Tx				
LiCl	0.23 ± 0.03	0.81 ± 0.17	0.53 ± 0.12	0.51 ± 0.07
Vehicle	0.22 ± 0.03	0.51 ± 0.05	0.47 ± 0.04	0.73 ± 0.07

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Alanine

Alanine is another non-essential amino acid. Alanine is primarily involved in protein synthesis, and little evidence exists for its involvement in psychiatric disorders. In the present set of experiments (Table A14), treatment with acute dextro-amphetamine did not significantly change alanine concentrations in the brain regions assessed, relative to vehicle. Similarly, treatment with antidepressants or anticonvulsants did not affect alanine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant decrease in alanine concentrations at 4 weeks in occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A14: Alanine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	0.42 ± 0.08	0.44 ± 0.05	0.85 ± 0.18	0.59 ± 0.04
Vehicle	0.42 ± 0.10	0.35 ± 0.02	0.48 ± 0.06	0.45 ± 0.07
Desipramine	0.39 ± 0.09	0.40 ± 0.05	0.72 ± 0.16	0.55 ± 0.08
Fluoxetine	0.32 ± 0.06	0.38 ± 0.03	0.60 ± 0.16	0.60 ± 0.06
Phenelzine	0.28 ± 0.06	0.64 ± 0.09	0.63 ± 0.10	0.43 ± 0.10
Vehicle	0.16 ± 0.02	0.47 ± 0.07	0.57 ± 0.07	0.35 ± 0.05
Lamotrigine	0.30 ± 0.07	0.44 ± 0.09	0.40 ± 0.06	0.32 ± 0.05
Sodium Valproate	0.36 ± 0.06	0.50 ± 0.15	0.37 ± 0.05	0.29 ± 0.03
Carbamazepine	0.25 ± 0.02	0.31 ± 0.02	0.30 ± 0.03	0.28 ± 0.02
Vehicle	0.24 ± 0.04	0.45 ± 0.10	0.33 ± 0.03	0.36 ± 0.10
1 Week Tx				
LiCl	0.34 ± 0.06	0.39 ± 0.10	0.85 ± 0.18	0.45 ± 0.04
Vehicle	0.31 ± 0.03	0.50 ± 0.10	1.52 ± 0.73	0.86 ± 0.19
2 Week Tx				
LiCl	0.23 ± 0.02	0.59 ± 0.07	0.59 ± 0.12	0.76 ± 0.15
Vehicle	0.18 ± 0.04	0.51 ± 0.10	0.38 ± 0.02	0.48 ± 0.04
4 Week Tx				
LiCl	0.26 ± 0.03	0.74 ± 0.13	0.56 ± 0.17	0.41 ± 0.05*
Vehicle	0.23 ± 0.03	0.50 ± 0.06	0.43 ± 0.03	0.60 ± 0.05

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Choline & Phosphorylcholine

Choline is an important component of cell membranes, and is a precursor of acetylcholine. As a result, choline has been implicated in the pathophysiology of mood disorders through several possible mechanisms (Duc et al. 1997; Gupta et al. 1995). Choline is also detectable at clinical field strengths. In the present set of experiments (Table A15), treatment with acute dextro-amphetamine did not significantly change choline concentration in the brain regions assessed, relative to vehicle. Data on phosphorylcholine was not quantified for the amphetamine-treated animals. Treatment with fluoxetine significantly increased phosphorylcholine, but not choline, concentration in the occipital cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect choline or phosphorylcholine concentration in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter choline or phosphorylcholine concentrations in the brain regions assessed, relative to vehicle. Clinical studies have also suggested that lithium does not affect choline concentrations in numerious brain regions of bipolar patients (Stoll et al. 1992; Bruhn et al. 1993; Kato et al. 1996; Ohara et al. 1998; Amaral et al. 2002; Wu et al. 2004; Brambilla et al. 2005).

Table A15: Choline and Phosphorylcholine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
CHOLINE				
d-amphetamine	0.26 ± 0.06	0.27 ± 0.03	0.52 ± 0.10	0.40 ± 0.02
Vehicle	0.27 ± 0.07	0.23 ± 0.01	0.32 ± 0.04	0.34 ± 0.06
Desipramine	0.17 ± 0.04	0.28 ± 0.04	0.42 ± 0.09	0.39 ± 0.06
Fluoxetine	0.16 ± 0.03	0.26 ± 0.02	0.40 ± 0.11	0.48 ± 0.04
Phenelzine	0.10 ± 0.02	0.39 ± 0.06	0.33 ± 0.05	0.24 ± 0.06
Vehicle	0.10 ± 0.02	0.33 ± 0.05	0.42 ± 0.07	0.31 ± 0.04
Lamotrigine	0.16 ± 0.04	0.36 ± 0.07	0.26 ± 0.04	0.27 ± 0.03
Sodium Valproate	0.22 ± 0.03	0.49 ± 0.17	0.28 ± 0.05	0.28 ± 0.02
Carbamazepine	0.15 ± 0.02	0.26 ± 0.03	0.22 ± 0.03	0.22 ± 0.04
Vehicle	0.16 ± 0.03	0.33 ± 0.07	0.24 ± 0.02	0.33 ± 0.10
1 Week Tx				
LiCl	0.19 ± 0.03	0.30 ± 0.08	0.55 ± 0.12	0.35 ± 0.03
Vehicle	0.16 ± 0.02	0.31 ± 0.05	0.91 ± 0.46	0.55 ± 0.09
2 Week Tx				
LiCl	0.12 ± 0.01	0.36 ± 0.04	0.34 ± 0.05	0.57 ± 0.11
Vehicle	0.10 ± 0.02	0.32 ± 0.06	0.24 ± 0.02	0.37 ± 0.03
4 Week Tx				
LiCl	0.15 ± 0.02	0.57 ± 0.11	0.38 ± 0.10	0.38 ± 0.06
Vehicle	0.14 ± 0.02	0.42 ± 0.05	0.30 ± 0.02	0.47 ± 0.04
PHOSPHORYLCHOLINE				
Desipramine	0.48 ± 0.10	0.39 ± 0.06	0.76 ± 0.13	0.72 ± 0.11
Fluoxetine	0.43 ± 0.08	0.35 ± 0.03	0.68 ± 0.18	0.80 ± 0.08*
Phenelzine	0.31 ± 0.06	0.54 ± 0.07	0.64 ± 0.10	0.45 ± 0.10
Vehicle	0.22 ± 0.03	0.44 ± 0.06	0.64 ± 0.10	0.43 ± 0.06
Lamotrigine	0.41 ± 0.09	0.45 ± 0.11	0.42 ± 0.07	0.40 ± 0.06
Sodium Valproate	0.57 ± 0.09	0.58 ± 0.19	0.46 ± 0.09	0.44 ± 0.04
Carbamazepine	0.39 ± 0.03	0.32 ± 0.04	0.33 ± 0.03	0.36 ± 0.05
Vehicle	0.38 ± 0.07	0.44 ± 0.09	0.40 ± 0.04	0.51 ± 0.17
1 Week Tx				
LiCl	0.36 ± 0.05	0.39 ± 0.14	0.79 ± 0.14	0.45 ± 0.06
Vehicle	0.35 ± 0.04	0.38 ± 0.06	1.61 ± 0.71	0.72 ± 0.11
2 Week Tx				
LiCl	0.30 ± 0.03	0.52 ± 0.08	0.49 ± 0.07	0.88 ± 0.18
Vehicle	0.22 ± 0.05	0.42 ± 0.09	0.32 ± 0.03	0.54 ± 0.04
4 Week Tx				
LiCl	0.32 ± 0.04	0.67 ± 0.11	0.48 ± 0.15	0.55 ± 0.09
Vehicle	0.30 ± 0.04	0.45 ± 0.06	0.42 ± 0.04	0.73 ± 0.08

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Threonine

Threonine is an essential amino acid. It is not known to have an effect in the brain (Young 1996), but it can be converted into serine or glycine. In the present set of experiments (Table A16), treatment with acute dextro-amphetamine significantly increased threonine concentrations in the temporal cortex, but not in other regions, relative to vehicle. Treatment with desipramine significantly increased threonine concentrations in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect threonine concentrations in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter threonine concentrations in the brain regions assessed, relative to vehicle.

Table A16: Threonine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.43 ± 0.11	0.34 ± 0.05	0.72 ± 0.12*	0.38 ± 0.04
Vehicle	0.41 ± 0.12	0.36 ± 0.04	0.35 ± 0.04	0.34 ± 0.04
Desipramine	0.31 ± 0.07*	0.26 ± 0.04	0.52 ± 0.11	0.45 ± 0.07
Fluoxetine	0.26 ± 0.04	0.25 ± 0.03	0.51 ± 0.10	0.48 ± 0.03
Phenelzine	0.15 ± 0.01	0.33 ± 0.02	0.29 ± 0.03	0.27 ± 0.08
Vehicle	0.11 ± 0.01	0.29 ± 0.05	0.48 ± 0.06	0.30 ± 0.04
Lamotrigine	0.19 ± 0.05	0.27 ± 0.04	0.28 ± 0.04	0.23 ± 0.04
Sodium Valproate	0.22 ± 0.04	0.44 ± 0.11	0.27 ± 0.05	0.25 ± 0.03
Carbamazepine	0.18 ± 0.02	0.19 ± 0.01	0.22 ± 0.02	0.22 ± 0.02
Vehicle	0.22 ± 0.04	0.30 ± 0.09	0.27 ± 0.02	0.32 ± 0.09
1 Week Tx				
LiCl	0.36 ± 0.08	0.17 ± 0.03	0.80 ± 0.14	0.20 ± 0.02
Vehicle	0.28 ± 0.02	0.20 ± 0.02	0.88 ± 0.36	0.26 ± 0.07
2 Week Tx				
LiCl	0.22 ± 0.03	0.22 ± 0.01	0.44 ± 0.11	0.38 ± 0.07
Vehicle	0.27 ± 0.01	0.19 ± 0.01	0.63 ± 0.20	0.27 ± 0.02
4 Week Tx				
LiCl	0.20 ± 0.03	0.28 ± 0.04	1.44 ± 0.93	0.19 ± 0.02
Vehicle	0.26 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.25 ± 0.03

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05

Acetate

Acetate is involved in the synthesis of numerous compounds, and has been found to decrease with age (Urenjak et al. 1993). Changes in acetate concentration have also been associated with changes in the concentration of NAA in multiple sclerosis (Davies et al. 1995), and may also indicate neuronal integrity. It has been found to increase in brain tumors (Florian et al. 1995) and abscesses (Kim et al. 1997). In the present set of experiments (Table A17), treatment with acute dextro-amphetamine did not significantly change acetate concentration in the brain regions assessed, relative to vehicle. Treatment with despiramine significantly increased acetate concentration in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect acetate concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase in acetate concentration at 2 weeks in the temporal cortex and a significant decrease at 4 weeks in the occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A17: Acetate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.19 ± 0.04	0.22 ± 0.04	0.45 ± 0.08	0.44 ± 0.04
Vehicle	0.29 ± 0.08	0.18 ± 0.01	0.30 ± 0.04	0.36 ± 0.06
Desipramine	0.16 ± 0.04*	0.20 ± 0.03	0.36 ± 0.08	0.27 ± 0.05
Fluoxetine	0.14 ± 0.02	0.18 ± 0.02	0.27 ± 0.08	0.28 ± 0.02
Phenelzine	0.09 ± 0.02	0.22 ± 0.03	0.19 ± 0.03	0.15 ± 0.03
Vehicle	0.06 ± 0.01	0.21 ± 0.03	0.22 ± 0.03	0.19 ± 0.03
Lamotrigine	0.12 ± 0.02	0.21 ± 0.05	0.19 ± 0.03	0.15 ± 0.01
Sodium Valproate	0.14 ± 0.04	0.24 ± 0.07	0.17 ± 0.03	0.15 ± 0.02
Carbamazepine	0.12 ± 0.02	0.16 ± 0.02	0.13 ± 0.01	0.15 ± 0.01
Vehicle	0.10 ± 0.03	0.18 ± 0.04	0.14 ± 0.01	0.20 ± 0.07
1 Week Tx				
LiCl	0.19 ± 0.02	0.18 ± 0.05	0.51 ± 0.10	0.28 ± 0.04
Vehicle	0.17 ± 0.02	0.22 ± 0.04	0.96 ± 0.45	0.43 ± 0.06
2 Week Tx				
LiCl	0.14 ± 0.01	0.26 ± 0.03	0.31 ± 0.04*	0.49 ± 0.10
Vehicle	0.11 ± 0.02	0.19 ± 0.04	0.20 ± 0.01	0.28 ± 0.02
4 Week Tx				
LiCl	0.12 ± 0.01	0.29 ± 0.06	0.26 ± 0.06	0.21 ± 0.03*
Vehicle	0.12 ± 0.02	0.21 ± 0.03	0.24 ± 0.02	0.33 ± 0.04

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Leucine

Leucine is an essential amino acid which has been linked to growth. This may relate to the fact that leucine is the most common amino acid found in proteins (Berg et al. 2002). Leucine also comprises an important class of transcription factor, the dimeric leucine zipper, which has been linked to processes involving circadian rhythm, learning and memory (Darlington et al. 1995; Yamaguchi et al. 2000; Sanyal et al. 2002). In the present set of experiments (Table A18), treatment with acute dextro-amphetamine did not significantly change leucine concentrations in the brain regions assessed, relative to vehicle. Similarly, treatment with antidepressants or anticonvulsants did not affect leucine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant decrease in leucine concentration at 1 week and at 4 weeks in the occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A18: Leucine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.13 ± 0.02	0.16 ± 0.02	0.24 ± 0.03	0.19 ± 0.02
Vehicle	0.14 ± 0.03	0.13 ± 0.01	0.16 ± 0.02	0.17 ± 0.02
Desipramine	0.11 ± 0.02	0.13 ± 0.02	0.22 ± 0.04	0.18 ± 0.03
Fluoxetine	0.11 ± 0.02	0.11 ± 0.01	0.20 ± 0.06	0.20 ± 0.02
Phenelzine	0.07 ± 0.01	0.15 ± 0.02	0.19 ± 0.03	0.11 ± 0.02
Vehicle	0.05 ± 0.00	0.13 ± 0.02	0.20 ± 0.02	0.12 ± 0.02
Lamotrigine	0.09 ± 0.02	0.17 ± 0.03	0.13 ± 0.02	0.09 ± 0.01
Sodium Valproate	0.12 ± 0.01	0.21 ± 0.06	0.15 ± 0.02	0.11 ± 0.01
Carbamazepine	0.10 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Vehicle	0.09 ± 0.02	0.15 ± 0.03	0.10 ± 0.01	0.12 ± 0.03
1 Week Tx				
LiCl	0.18 ± 0.03	0.18 ± 0.05	0.42 ± 0.10	0.27 ± 0.02*
Vehicle	0.18 ± 0.02	0.23 ± 0.04	0.88 ± 0.45	0.51 ± 0.09
2 Week Tx				
LiCl	0.12 ± 0.01	0.23 ± 0.03	0.28 ± 0.06	0.44 ± 0.08
Vehicle	0.11 ± 0.02	0.19 ± 0.03	0.17 ± 0.01	0.25 ± 0.02
4 Week Tx				
LiCl	0.11 ± 0.01	0.33 ± 0.06	0.26 ± 0.07	0.23 ± 0.03*
Vehicle	0.12 ± 0.02	0.26 ± 0.03	0.23 ± 0.01	0.35 ± 0.03

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Nicotinamide

Nicotinamide, or vitamin B3, plays an integral role in cellular energy metabolism. It has been found to be concentrated primarily in the basal ganglia and thalamus (Baker et al. 1984). Treatment with nicotinamide has been found to reduce hypoxic ischemic brain injury in adult and newborn rats via prevention in oxidative stress (Feng et al. 2006). There is little evidence suggesting involvement of nicotinamide in psychiatric disorders. Data on nicotinamide was not quantified for the amphetamine-treated animals. In the present set of experiments (Table A19), treatment with antidepressants or anticonvulsants did not affect nicotinamide concentrations in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter nioctinamide concentrations in the brain regions assessed, relative to vehicle.

Table A19: Nicotinamide Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
Desipramine	0.06 ± 0.01	0.10 ± 0.01	0.18 ± 0.04	0.14 ± 0.02
Fluoxetine	0.07 ± 0.01	0.09 ± 0.00	0.17 ± 0.05	0.16 ± 0.01
Phenelzine	0.06 ± 0.01	0.12 ± 0.02	0.14 ± 0.02	0.09 ± 0.02
Vehicle	0.06 ± 0.01	0.13 ± 0.02	0.16 ± 0.02	0.12 ± 0.02
Lamotrigine	0.07 ± 0.02	0.12 ± 0.03	0.11 ± 0.01	0.09 ± 0.01
Sodium Valproate	0.08 ± 0.01	0.16 ± 0.05	0.10 ± 0.01	0.10 ± 0.01
Carbamazepine	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.00
Vehicle	0.06 ± 0.01	0.12 ± 0.03	0.08 ± 0.01	0.11 ± 0.03
1 Week Tx				
LiCl	0.10 ± 0.02	0.16 ± 0.05	0.30 ± 0.05	0.16 ± 0.01
Vehicle	0.11 ± 0.02	0.18 ± 0.04	0.48 ± 0.20	0.28 ± 0.06
2 Week Tx				
LiCl	0.08 ± 0.01	0.18 ± 0.02	0.22 ± 0.06	0.26 ± 0.04
Vehicle	0.07 ± 0.01	0.18 ± 0.03	0.19 ± 0.01	0.19 ± 0.02
4 Week Tx				
LiCl	0.08 ± 0.01	0.26 ± 0.04	0.24 ± 0.06	0.19 ± 0.03
Vehicle	0.07 ± 0.01	0.18 ± 0.03	0.18 ± 0.01	0.21 ± 0.02

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

Formate

There is no evidence suggesting involvement of formate in psychiatric disorders. In the present set of experiments (Table A20), treatment with acute dextroamphetamine did not significantly change formate concentrations in the brain regions assessed, relative to vehicle. Similarly, treatment with antidepressants or anticonvulsants did not affect formate concentrations in the brain regions assessed, relative to vehicle. One, 2 and 4 week treatment with lithium did not alter formate concentrations in the brain regions assessed, relative to vehicle.

Table A20: Formate Concentrations (µmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.06 ± 0.02	0.08 ± 0.01	0.14 ± 0.02	0.16 ± 0.04
Vehicle	1.17 ± 0.87	0.13 ± 0.05	0.13 ± 0.02	0.13 ± 0.02
Desipramine	0.10 ± 0.02	0.03 ± 0.00	0.11 ± 0.05	0.04 ± 0.01
Fluoxetine	0.13 ± 0.02	0.03 ± 0.00	0.06 ± 0.01	0.05 ± 0.00
Phenelzine	0.08 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Vehicle	0.08 ± 0.02	0.04 ± 0.00	0.07 ± 0.01	0.04 ± 0.00
Lamotrigine	0.13 ± 0.03	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.00
Sodium Valproate	0.13 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
Carbamazepine	0.10 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Vehicle	0.09 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.02
1 Week Tx				
LiCl	0.12 ± 0.02	0.11 ± 0.02	0.18 ± 0.03	0.10 ± 0.02
Vehicle	0.11 ± 0.01	0.10 ± 0.02	0.22 ± 0.09	0.13 ± 0.02
2 Week Tx				
LiCl	0.10 ± 0.01	0.09 ± 0.02	0.10 ± 0.01	0.11 ± 0.01
Vehicle	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.01	0.10 ± 0.02
4 Week Tx				
LiCl	0.07 ± 0.01	0.10 ± 0.03	0.09 ± 0.02	0.07 ± 0.01
Vehicle	0.07 ± 0.00	0.11 ± 0.02	0.08 ± 0.01	0.10 ± 0.01

Note: Concentrations are reported as µmol/g wet weight (mean ± SEM).

Valine

Valine is also an essential amino acid. It is linked to several human disorders by its substitution in protein sequences linked to attention deficit hyperactivity disorder (Mazei-Robison et al. 2005) and sickle cell anemia. In the present set of experiments (Table A21), treatment with acute dextro-amphetamine significantly increased valine concentrations in the temporal cortex, but not in other regions, relative to vehicle. Treatment with antidepressants or anticonvulsants did not affect valine concentration in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a strong trend toward decreased valine concentrations in the occipital cortex, but this only reached significance at 4 weeks, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A21: Valine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	**Hippocampus**	**Temporal Cortex**	**Occipital Cortex**
d-amphetamine	0.07 ± 0.02	0.08 ± 0.01	0.16 ± 0.02*	0.11 ± 0.01
Vehicle	0.08 ± 0.02	0.06 ± 0.00	0.10 ± 0.01	0.09 ± 0.02
Desipramine	0.07 ± 0.02	0.08 ± 0.01	0.12 ± 0.03	0.10 ± 0.02
Fluoxetine	0.06 ± 0.01	0.07 ± 0.00	0.12 ± 0.04	0.11 ± 0.01
Phenelzine	0.04 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.06 ± 0.01
Vehicle	0.03 ± 0.00	0.08 ± 0.01	0.11 ± 0.01	0.07 ± 0.01
Lamotrigine	0.04 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.05 ± 0.00
Sodium Valproate	0.06 ± 0.01	0.10 ± 0.04	0.08 ± 0.01	0.06 ± 0.00
Carbamazepine	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Vehicle	0.05 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.02
1 Week Tx				
LiCl	0.08 ± 0.01	0.08 ± 0.02	0.19 ± 0.04	0.11 ± 0.01
Vehicle	0.07 ± 0.01	0.10 ± 0.02	0.37 ± 0.18	0.20 ± 0.04
2 Week Tx				
LiCl	0.05 ± 0.00	0.11 ± 0.01	0.11 ± 0.03	0.17 ± 0.03
Vehicle	0.04 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.00
4 Week Tx				
LiCl	0.04 ± 0.00	0.13 ± 0.02	0.09 ± 0.02	0.09 ± 0.01*
Vehicle	0.05 ± 0.01	0.10 ± 0.02	0.09 ± 0.00	0.14 ± 0.01

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Succinate

Succinate is a component of the citric acid (or Krebs) cycle, and can also donate electrons to the mitochondrial electron transport chain to produce ATP (Berg et al. 2002). There is no evidence suggesting involvement of succinate in psychiatric disorders, but as evidence for mitochondrial involvement in psychiatric disorders, including bipolar disorder (Stork and Renshaw 2005), accumulates this may change. In the present set of experiments (Table A22), treatment with acute dextro-amphetamine did not significantly change succinate concentrations in the brain regions assessed, relative to vehicle. Treatment with despiramine significantly decreased succinate concentrations in the hippocampus, but not in other regions, relative to vehicle. There was no effect of the other antidepressants in any of the regions assessed. Treatment with all anticonvulsants tested significantly decreased succinate concentrations in both the occipital cortex and hippocampus, but not in other regions, relative to vehicle. Lamotrigine also decreased succinate concentrations in the temporal cortex, relative to vehicle. None of the anticonvulsants altered succinate concentration in the pre-frontal cortex. One, 2 and 4 week treatment with lithium did not alter succinate concentrations in the brain regions assessed, relative to vehicle.

Table A22: Succinate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.04 ± 0.01
Vehicle	0.05 ± 0.01	0.02 ± 0.00	0.08 ± 0.01	0.07 ± 0.02
Desipramine	0.00 ± 0.00	0.05 ± 0.01*	0.03 ± 0.01	0.03 ± 0.01
Fluoxetine	0.00 ± 0.00	0.07 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Phenelzine	0.01 ± 0.00	0.08 ± 0.02	0.07 ± 0.02	0.04 ± 0.01
Vehicle	0.01 ± 0.00	0.11 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Lamotrigine	0.01 ± 0.00	0.02 ± 0.00**	0.02 ± 0.01	0.02 ± 0.00**
Sodium Valproate	0.01 ± 0.00	0.04 ± 0.01**	0.03 ± 0.00	0.03 ± 0.01*
Carbamazepine	0.01 ± 0.00	0.06 ± 0.01*	0.04 ± 0.01	0.04 ± 0.01*
Vehicle	0.02 ± 0.00	0.12 ± 0.02	0.06 ± 0.01	0.09 ± 0.02
1 Week Tx				
LiCl	0.01 ± 0.00	0.04 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Vehicle	0.01 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
2 Week Tx				
LiCl	0.00 ± 0.00	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Vehicle	0.00 ± 0.00	0.08 ± 0.02	0.04 ± 0.01	0.05 ± 0.00
4 Week Tx				
LiCl	0.01 ± 0.00	0.08 ± 0.02	0.02 ± 0.00	0.02 ± 0.00
Vehicle	0.00 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05, **p<0.005

Fumarate

Fumarate is formed by the oxidation of succinate, and has no reported involvement in psychiatry disorders. In the present set of experiments (Table A23), treatment with acute dextro-amphetamine showed a strong trend toward an increase in fumarate, but this did not reach statistical significance for any region. Treatment with either despiramine significantly increased fumarate concentrations in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect of the other antidepressants in any of the regions assessed. Treatment with anticonvulsants did not affect fumarate concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant decrease in fumarate concentration at 1 week in the occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A23: Fumarate Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.09 ± 0.02	0.08 ± 0.00	0.09 ± 0.02	0.11 ± 0.01
Vehicle	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Desipramine	0.06 ± 0.01*	0.04 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
Fluoxetine	0.06 ± 0.01	0.04 ± 0.00	0.07 ± 0.02	0.08 ± 0.01
Phenelzine	0.02 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Vehicle	0.02 ± 0.00	0.06 ± 0.01	0.08 ± 0.01	0.05 ± 0.01
Lamotrigine	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.03 ± 0.00
Sodium Valproate	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.01
Carbamazepine	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Vehicle	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01
1 Week Tx				
LiCl	0.04 ± 0.00	0.04 ± 0.02	0.08 ± 0.01	0.06 ± 0.00*
Vehicle	0.04 ± 0.01	0.06 ± 0.01	0.13 ± 0.03	0.12 ± 0.02
2 Week Tx				
LiCl	0.04 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02
Vehicle	0.03 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.08 ± 0.01
4 Week Tx				
LiCl	0.03 ± 0.00	0.08 ± 0.01	0.09 ± 0.02	0.06 ± 0.00
Vehicle	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.08 ± 0.01

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

Uracil

Uracil is a pyrimidine, and is one of the four base pairs found in ribonucleic acid, binding with adenine (Berg et al. 2002). Its involvement in psychiatric disorders is not established. Data on uracil were not quantified for the amphetamine-treated animals. In the present set of experiments (Table A24), treatment with anticonvulsants did not affect uracil concentrations in the brain regions assessed, relative to vehicle. Treatment with despiramine significantly increased uracil concentrations in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. One, 2 and 4 week treatment with lithium did not alter uracil concentrations in the brain regions assessed, relative to vehicle.

Table A24: Uracil Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
Desipramine	0.05 ± 0.01*	0.04 ± 0.00	0.10 ± 0.03	0.07 ± 0.01
Fluoxetine	0.03 ± 0.01	0.03 ± 0.00	0.08 ± 0.02	0.06 ± 0.01
Phenelzine	0.03 ± 0.00	0.03 ± 0.00	0.06 ± 0.01	0.03 ± 0.01
Vehicle	0.02 ± 0.00	0.04 ± 0.01	0.06 ± 0.01	0.03 ± 0.00
Lamotrigine	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
Sodium Valproate	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.00
Carbamazepine	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Vehicle	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
1 Week Tx				
LiCl	0.04 ± 0.00	0.07 ± 0.02	0.14 ± 0.03	0.06 ± 0.00
Vehicle	0.04 ± 0.00	0.07 ± 0.01	0.29 ± 0.14	0.10 ± 0.02
2 Week Tx				
LiCl	0.05 ± 0.00	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.02
Vehicle	0.04 ± 0.00	0.08 ± 0.01	0.06 ± 0.00	0.07 ± 0.00
4 Week Tx				
LiCl	0.04 ± 0.00	0.11 ± 0.02	0.08 ± 0.02	0.09 ± 0.04
Vehicle	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.09 ± 0.01

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05

Histidine

Histidine is an essential amino acid, and a protein building block. There is no evidence suggesting involvement of histidine in psychiatric disorders. Data on histidine were not quantified for the amphetamine-treated animals. In the present set of experiments (Table A25), treatment with anticonvulsants did not affect histidine concentrations in the brain regions assessed, relative to vehicle. Treatment with either despiramine or fluoxetine significantly increased histidine concentrations in the pre-frontal cortex, but not in other regions, relative to vehicle. There was no effect from the other antidepressants in any of the regions assessed. Lithium-treated rats showed a significant decrease in histidine concentration at 2 weeks in the temporal cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A25: Histidine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
Desipramine	0.03 ± 0.00**	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Fluoxetine	0.03 ± 0.00**	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01
Phenelzine	0.02 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Vehicle	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Lamotrigine	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
Sodium	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Valproate	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Carbamazepine	0.02 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Vehicle				
1 Week Tx				
LiCl	0.02 ± 0.00	0.06 ± 0.01	0.62 ± 0.21	0.07 ± 0.01
Vehicle	0.06 ± 0.03	0.09 ± 0.01	0.76 ± 0.53	0.10 ± 0.02
2 Week Tx				
LiCl	0.04 ± 0.02	0.08 ± 0.02	0.70 ± 0.26*	0.12 ± 0.02
Vehicle	0.03 ± 0.00	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.01
4 Week Tx				
LiCl	0.02 ± 0.00	0.10 ± 0.01	0.50 ± 0.14	0.08 ± 0.02
Vehicle	0.03 ± 0.00	0.08 ± 0.01	0.26 ± 0.11	0.09 ± 0.01

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*p<0.05, **p<0.005

Isoleucine

Isoleucine is also an essential amino acid. It is an isomer of leucine, but does not have established links to psychiatric disorders. In the present set of experiments (Table A26), treatment with acute dextro-amphetamine significantly increased isoleucine concentrations in the temporal cortex, but not in other regions, relative to vehicle. Treatment with antidepressants or anticonvulsants did not affect isoleucine concentrations in the brain regions assessed, relative to vehicle. Lithium-treated rats showed a significant increase in isoleucine concentration at 2 weeks and a decrease at 4 weeks in the occipital cortex, relative to vehicle. There were no other differences across brain regions and treatment lengths.

Table A26: Isoleucine Concentrations (μmol/gram wet weight) across Four Brain Regions, for Medication-Treated relative to Vehicle-Treated Rats.

	Pre-Frontal Cortex	Hippocampus	Temporal Cortex	Occipital Cortex
d-amphetamine	0.04 ± 0.01	0.04 ± 0.01	0.09 ± 0.01*	0.07 ± 0.01
Vehicle	0.06 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.06 ± 0.01
Desipramine	0.05 ± 0.01	0.05 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
Fluoxetine	0.04 ± 0.01	0.04 ± 0.00	0.08 ± 0.03	0.07 ± 0.01
Phenelzine	0.03 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Vehicle	0.02 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.04 ± 0.01
Lamotrigine	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
Sodium Valproate	0.04 ± 0.00	0.06 ± 0.02	0.06 ± 0.01	0.04 ± 0.00
Carbamazepine	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Vehicle	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
1 Week Tx				
LiCl	0.04 ± 0.00	0.09 ± 0.02	0.18 ± 0.05	0.28 ± 0.04
Vehicle	0.06 ± 0.01	0.10 ± 0.02	0.75 ± 0.37	0.21 ± 0.03
2 Week Tx				
LiCl	0.04 ± 0.01	0.10 ± 0.01	0.16 ± 0.03	0.41 ± 0.10*
Vehicle	0.04 ± 0.01	0.07 ± 0.01	0.15 ± 0.04	0.13 ± 0.01
4 Week Tx				
LiCl	0.04 ± 0.00	0.14 ± 0.02	0.25 ± 0.07	0.11 ± 0.01*
Vehicle	0.05 ± 0.01	0.09 ± 0.01	0.24 ± 0.03	0.15 ± 0.01

Note: Concentrations are reported as μmol/g wet weight (mean ± SEM).

*$p<0.05$

A.4 Conclusions

High field NMR spectroscopy is a useful tool for the study of brain neurochemistry and drug effects. The drugs assessed here appear to have differing effects on many of the metabolites examined. Many of these effects have not been examined for clinical relevance, and further, hypothesis-driven investigation is warranted. Interestingly, while many metabolites are NMR visible, these are largely restricted to compounds of relatively simple chemical composition. The most readily detectable appear to be the amino acids. Moreover, the metabolites that have received the most research attention are those that are easily detectable at lower field strengths, typical of most clinical *in vivo* investigations.

A.5 Bibliography

Amaral JAMS, Lafer B, Tamada RS, Issler CK, Cerri GG, de Castro CC. A ^{1}H MRS study of the anterior cingulated gyrus in euthymic bipolar patients taking lithium. Biol Psychiatry 2002;51:87S.

Anderzhanova E, Rayevsky KS, Saransaari P, Riitamaa E, Oja SS. Effects of sydnocarb and d-amphetamine on extracellular levels of amino acids in the rat caudate-putamen. Eur J Pharmacol 2001;428:87-95.

Anderzhanova E, Rayevsky KS, Saransaari P, Riitamaa E, Oja SS. Effects of acute toxic doses of psychostimulants on extracellular levels of excitatory amino acids and taurine in rats. Comparison of d-amphetamine and sydnocarb. Ann NY Acad Sci 2002;965:193-203.

Andiarzhanova EA, Saransaari R, Riitamaa E, Oiia SS, Raevskii KS. Extracellular level of neuroactive amino acids in the rat neostriatum after treatment with psychostimulants (microdialysis study). Eksp Klin Farmakol 2002;65:7-13.

Baker H, Frank O, Chen T, Feingold S, DeAngelis B, Baker E. Vitamin content of some normal human brain segments. J Neurosci Res 1984;11:419-435.

Berg JM, Stryer L, Tymoczko JL. Biochemistry, 5th Edition. WH Freeman: New York, NY. 2002.

Bertolino A, Frye M, Callicott JH, Mattay VS, Rakow R, Shelton-Repella J, Post R, Weinberger DR. Neuronal pathology in the hippocampal area of patients with bipolar disorder: A study with proton magnetic resonance spectroscopic imaging. Biol Psychiatry 2003;53:906-913.

Bertolino A, Roffman JL, Lipska BK, van Gelderen P, Olson A, Weinberger DR. Reduced N-acetylasparate in prefrontal cortex of adult rats with neonatal hippocampal damage. Cereb Cortex 2002;12:983-990.

Betz H, Kuhse J, Schmieden B, Laube B, Kirsch J, Harvey RJ. Structure and functions of inhibitory and excitatory glycine receptors. Ann NY Acad Sci 1999;868:667-676.

Bhagwagar Z, Wylezinska M, Taylor M, Jezzard P, Matthews PM, Cowen PJ. Increased brain GABA concentrations following acute administration of a selective serotonin reuptake inhibitor. Am J Psychiatry 2004;161:368-370.

Brambilla P, Stanley JA, Nicoletti MA, Sassi RB, Mallinger AG, Frank E, Kupfer D, Keshavan MS, Soares JC. ^{1}H magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in bipolar disorder patients. J Affect Disord 2005;86:61-67.

Brambilla P, Stanley JA, Sassi RB, Nicoletti MA, Mallinger AG, Keshavan MS, Soares JC. ^{1}H MRS study of the dorsolateral prefrontal cortex in healthy individuals before and after lithium administration. Neuropsychopharmacol 2004;29:1918-1924.

Bruhn H, Stoppe G, Staedt J, Merboldt KD, Hanicke W, Frahm J. Quantitative proton MRS in vivo shows cerebral myo-inositol and cholines to be unchanged in manic-depressive patients treated with lithium. Proc Soc Magn Reson Med 1993:1543.

Castel-Branco M, Lebre V, Falcao A, Figueiredo I, Caramona M. Relationship between plasma and brain levels and the anticonvulsant effect of lamotrigine in rats. Eur J Pharmacol 2003;482:163-168.

Chang L, Cloak CC, Ernst T. Magnetic resonance spectroscopy studies of GABA in neuropsychiatric disorders. J Clin Psychiatry 2003;64(Suppl 3):7-14.

Chen G, Huang LD, Jiang YM, Manji HK. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. J Neurochem 1999;72:879–882.

Choe ES, Chung KT, Mao L, Wang JQ. Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors. Neuropsychopharmacol 2002;27:565-575.

Choi K-H, Clements RLH, Greenshaw AJ. Simultaneous AMPA/kainate receptor blockade and dopamine D2/3 receptor stimulation in the nucleus accumbens decreases brain stimulation reward in rats. Behav Brain Res 2005;158:79-88.

Darlington GJ, Wang N, Hanson RW. C/EBP alpha: A critical regulator of genes governing integrative metabolic processes. Curr Opin Genet Dev 1995;5:565-570.

Davies SE, Newcombe J, Williams SR, McDonald WI, Clark JB. High resolution proton NMR spectroscopy of multiple sclerosis lesions. J Neurochem 1995;64:742-748.

Deicken RF, Eliaz Y, Feiwell R, Schuff N. Increased thalamic N-acetylaspartate in male patients with familial bipolar I disorder. Psychiatry Res Neuroimag 2001;106:35-45.

de Koning TJ. Treatment with amino acids in serine deficiency disorders. J Inherit Metab Dis 2006;29:347-351.

Del Arco A, Martinez R, Mora F. Amphetamine increases extracellular concentrations of glutamate in the prefrontal cortex of the awake rat: A microdialysis study. Neurochem Res 1998;23:1153-1158.

Duc CO, Weber Ah, Trabesinger AH, Meier D, Boesiger P. Recycling the cholines. Int Soc Magn Reson Med 1997;6:1210.

Dwivedi Y, Agrawal AK, Rizavi HS, Pandey GN. Antidepressants reduce phosphoinositide-specific phospholipase C (PI-PLC) activity and the mRNA and protein expression of selective PLC β1 isozyme in rat brain. Neuropharmacol 2002;43:1269-1279.

Epperson CN, Gueorguieva R, Czarkowski KA, Stiklus S, Sellers E, Krystal JH, Rothman DL, Mason GF. Preliminary evidence of reduced occipital GABA concentrations in puerperal women: A ^{1}H-MRS study. Psychopharmacol 2006;186:425-433.

Feldman RS, Meyer JS, Quenzer LF. Principles of Neuropsychopharmacology. Sinauer Associates Inc: Cambridge, MA. 1997.

Feng Y, Paul IA, LeBlanc MH. Nicotinamide reduces hypoxic ischemic brain injury in the newborn rat. Brain Res Bull 2006;69:117-122.

Florian CL, Preece NE, Bhakoo KK, Williams SR, Noble M. Characteristic metabolic profiles revealed by ^{1}H NMR spectroscopy for three types of human brain and nervous system tumours. NMR Biomed 1995;8:253-264.

Gaull GE. Taurine as a conditionally essential nutrient in man. J Am Coll Nutr 1986;5:121-125.

Gaull GE. Taurine in pediatric nutrition: Review and update. Pediatrics 1989;83:433-442.

Ghoshdastidar D, Dutta RN, Poddar MK. In vivo distribution of lithium in plasma and brain. Ind J Exp Biol 1989;27:950-954.

Goddard AW, Mason GF, Almai A, Rothman DL, Behar KL, Petroff OA, Charney DS, Krystal JH. Reductions in occipital cortex GABA levels in panic disorder detected with ^{1}H-magnetic resonance spectroscopy. Arch Gen Psychiatry 2001;58:556-561.

Gottesfeld Z. Effect of lithium and other alkali metals on brain chemistry and behavior: Glutamic acid and GABA in brain regions. Psychopharmacologia 1976;45:239-242.

Gupta RK, Bhatia V, Poptani H, Gujral RB. Brain metabolic changes on in vivo proton magnetic resonance spectroscopy in children with congenital hypothyroidism. J Pediatr 1995;126:389-392.

Hamakawa H, Kato T, Shioiri T, Inubushi T, Kato, N. Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. Psychol Med 1999;29:639-644.

Han X, Patters AB, Jones DP, Zelikovic I, Chesney RW. The taurine transporter: Mechanisms of regulation. Acta Physiol 2006;187:61-73.

Hardy DL, Norwood TJ. Spectral editing techniques for the in vitro and in vivo detection of taurine. J Magn Reson 1998;133:70-78.

Hasegawa H, Osada K, Misonoo A, Morinobu S, Yamamoto H, Miyamoto E, Asakura M. Chronic carbamazepine treatment increases myristoylated alanine-rich C kinase substrate phosphorylation in the rat cerebral cortex via down-regulation of calcineurin Aα. Brain Res 2003;994:19-26.

Helms G, Ciumas C, Kyaga S, Savic I. Increased thalamus levels of glutamate and glutamine (Glx) in patients with idiopathic generalized epilepsy. J Neurol Neurosurg Psychiatry 2006;77:489-494.

Heresco-Levy U. Amino acid transmitter systems. In: Biological Psychiatry, 1st Edition. D'haenen H, den Boer JA, Willner P (eds.). John Wiley & Sons: London, UK. 2002.

Jope RS, Miller JM, Ferraro TN, Hare TA. Chronic lithium treatment and status epilepticus induced by lithium and pilocarpine cause selective changes in amino acid concentrations in rat brain regions. Neurochem Res 1989;14:829-834.

Kato T, Hamakawa H, Shioiri T, Murashita J, Takahashi Y, Takahashi S, Inubushi T. Choline-containing compounds detected by proton magnetic resonance spectroscopy in the basal ganglia in bipolar disorder. J Psychiatry Neurosci 1996;21:248-254.

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, and others. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded ^{31}P-MRS. Psychol Med 1995;25:557-566.

Keck ME, Strohle A. Challenge studies in anxiety disorders. Handb Exp Pharmacol 2005;169:449-468.

Kim SH, Chang KH, Song IC, Han MH, Kim HC, Kang HS, Han MC. Brain abscess and brain tumor: Discrimination with in vivo H-1 MR spectroscopy. Radiol 1997;204:239-245.

Klunk WE, Xu C, Panchalingam K, McClure RJ, Pettegrew JW. Quantitative ^{1}H and ^{31}P MRS of PCA extracts of postmortem Alzheimer's disease brain. Neurobiol Aging 1996;17:349-357.

Kovacs Z, Kekesi KA, Bobest M, Torok T, Szilagyi N, Szikra T, Szepesi Z, Nyilas R, Dobolyi A, Palkovits M, Juhasz G. Post mortem degradation of nucleosides in the brain: Comparison of human and rat brains for estimation of in vivo concentration of nucleosides. J Neurosci Methods 2005;148:88-93.

Kreis R. Quantitative localized ^{1}H MR spectroscopy for clinical use. Prog Magn Reson Spectroscopy 1997;31:155-195.

Kugaya A, Sanacora G. Beyond monoamines: Glutamatergic function in mood disorders. CNS Spectr 2005;10:808-819.

Laidlaw SA, Kopple JD. Newer concepts of the indispensable amino acids. Am J Clin Nutr 1987;46:593-605.

Malhi GS, Valenzuela M, Wen W, Sachidev P. Magnetic resonance spectroscopy and its applications in psychiatry. Aust NZ J Psychiatry 2002;36:31-43.

Marcus SR, Nadiger HA, Chandrakala MV, Rao TI, Sadasivudu B. Acute and short-term effects of lithium on glutamate metabolism in rat brain. Biochem Pharmacol 1986;35:365-369.

Mazei-Robison MS, Couch RS, Shelton RC, Stein MA, Blakely RD. Sequence variation in the human dopamine transporter gene in children with attention deficit hyperactivity disorder. Neuropharmacol 2005;49:724-736.

Mcgeehan AJ, Janak PH, Olive MF. Effect of the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) on the acute locomotor stimulant properties of cocaine, D-amphetamine, and the dopamine reuptake inhibitor GBR12909 in mice. Psychopharmacol 2004;174:266-273.

Michaelis T, Merboldt KD, Bruhn H, Hanicke W, Frahm J. Absolute concentrations of metabolites in the adult human brain in vivo: Quantification of localized proton MR spectra. Radiol 1993;187:219-227.

Nanitsos EK, Nguyen KT, St'astny F, Balcar VJ. Glutamatergic hypothesis of schizophrenia: Involvement of Na+/K+-dependent glutamate transport. J Biomed Sci 2005;12:975-984.

Nicklas WJ, Zeevalk G, Hyndman A. Interactions between neurons and glia in glutamate/glutamine compartmentation. Biochem Soc Transact 1987;15:208-213.

Nordahl TE, Salo R, Natsuaki Y, Galloway GP, Waters C, Moore CD, Kile S, Buonocore MH. Methamphetamine users in sustained abstinence: A proton magnetic resonance spectroscopy study. Arch Gen Psychiatry 2005;62:444-452.

Nordahl TE, Salo R, Possin K, Gibson DR, Flynn N, Leamon M, Galloway GP, Pfefferbaum A, Spielman DM, Adalsteinsson E, Sullivan EV. Low N-acetyl-aspartate and high choline in the anterior cingulum of recently abstinent methamphetamine-dependent subjects: A preliminary proton MRS study. Magnetic resonance spectroscopy. Psychiatry Res 2002;116:43-52.

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH. Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Res 2000;880:84-91.

Ohara K, Isoda H, Suzuki Y, Takehara Y, Ochiai M, Takeda H, Igarashi Y, Ohara K. Proton magnetic resonance spectroscopy of the lenticular nuclei in bipolar I affective disorder. Psychiatry Res 1998;84:55-60.

Peng L, Hertz L, Huang R, Sonnewald U, Petersen SB, Westergaard N, Larsson O, Schousboe A. Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. Develop Neurosci 1993;15:367-377.

Petroff OAC, Spencer DD, Alger JR, Prichard JW. High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. Neurol 1989;39:1197-1202.

Plenge P. Acute lithium effects on rat brain glucose metabolism in vivo. Int Pharmacopsychiatry 1976;260:587-596.

Rudkin TM, Arnold DL. Proton magnetic resonance spectroscopy for the diagnosis and management of cerebral disorders. Arch Neurol 1999;56:919-926.

Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA, Berman RM, Charney DS, Krystal JH. Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. Arch Gen Psychiatry 1999;56:1043-1047.

Saunders DE, Howe FA, van den Boogaart A, Griffiths JR, Brown MM. Aging of the adult human brain: In vivo quantitation of metabolite content with proton magnetic resonance spectroscopy. J Magn Reson Imag 1999;9:711-716.

Sanyl S, Sandstrom DJ, Hoeffer CA, Ramaswami M. AP-1 functions upstream of CREB to control synaptic plasticity in Drosophila. Nature 2002;416:870-874.

Seegmiller JE, Rosenbloom FM, Kelley WN. Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. Science 1967;155:1682.

Siegel GJ, Agranoff BW, Albers RW, Molinoff P (eds.). Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Raven Press: New York, NY. 1989.

Stoll AL, Renshaw PF, Sachs GS, Guimaraes AR, Miller C, Cohen BM, Lafer B, Gonzalez RG. The human brain resonance of choline-containing compounds is similar in patients receiving lithium treatment and controls: An in vivo proton magnetic resonance spectroscopy study. Biol Psychiatry 1992;32:944-949.

Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: Evidence from magnetic resonance spectroscopy research. Mol Psychiatry 2005;10:900-919.

Tan J, Bluml S, Hoang T, Dubowitz D, Mevenkamp G, Ross B. Lack of effect of oral choline supplement on the concentrations of choline metabolites in human brain. Magn Reson Med 1998;39:1005-1010.

Thomson AM. Glycine modulation of the NMDA receptor/channel complex. Trends Neurosci 1989;12:349-353.

Tsai G, Coyle JT. N-Acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531-540.

Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J Neurosci 1993;13:981-989.

van Zijl PC, Barker PB. Magnetic resonance spectroscopy and spectroscopic imaging for the study of brain metabolism. Ann NY Acad Sci 1997;820:75-96.

van Zijl PCM, Moonen CTW. In situ changes in purine nucleotide and N-acetyl concentrations upon inducting global ischemia in cat brain. Magn Reson Med 1993;29:381-385.

Veech RL. The metabolism of lactate. NMR Biomed 1991;4:53-58.

Wu RH, O'Donnell T, Ulrich M, Asghar SJ, Hanstock CC, Silverstone PH. Brain choline concentrations may not be altered in euthymic bipolar disorder patients chronically treated with either lithium or sodium valproate. Ann Gen Hosp Psychiatry 2004;3:13.

Yamaguchi S, Mitsui S, Yan L, Yagita K, Miyake S, Okamura H. Role of DBP in the circadian oscillatory mechanism. Mol Cell Biol 2000;20:4773-4781.

Young SN. Behavioural effects of dietary neurotransmitter precursors: Basic and clinical aspects. Neurosci Biobehav Rev 1996;20:313-323.

Yuzaki M, Forrest D, Curran T, Connor JA. Selective activation of calcium permeability by aspartate in purkinje cells. Science 1996;273:1112-1114.

Appendix B: Drug Preparation for Animal Experimentation

Where applicable, drug doses were based on free base weights. The table below outlines all drugs used and their conversion factors.

Drug	Chemical Formula	Molecular Weight	Formula Weight	Conversion Factor
Lithium Chloride	$LiCl$	42.39	42.39	n/a
Dextro-amphetamine sulfate	$C_{18}H_{26}N_2\cdot H_2O_4S$	270.41	368.49	0.734
Phenelzine sulfate	$C_8H_{12}N_2\cdot H_2O_4S$	136.22	234.30	0.581
Fluoxetine hydrochloride	$C_{17}H_{18}F_3NO\cdot HCl$	309.36	345.79	0.895
Desipramine hydrochloride	$C_{18}H_{22}N_2\cdot HCl$	266.42	302.90	0.880
Lamotrigine	$C_9H_7Cl_2N_5$	256.09	256.09	n/a
Carbamazepine	$C_{15}H_{12}N_2O$	236.27	236.27	n/a
Sodium Valproate	$C_8H_{15}NaO_2$	143.21	166.20	0.862

All compounds can be stored at room temperature and solutions should be made fresh daily.

Example: The following calculation illustrates how to determine the amount of compound to add based on the desired free base drug dose:

- For a desired dose of 10mg of phenelzine free base, 17.21 mg of phenelzine sulfate (i.e. 10 mg free base ÷ 0.581) must be administered.

Appendix C: Standard Operating Procedures for Animal Experimentation

C.1 Introduction

All procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care, and were reviewed and approved by the local Animal Policy and Welfare Committee. All experiments were reviewed and approved by the local Animal Policy and Welfare Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

C.2 Treatment

Adult male Sprague-Dawley rats, were housed in pairs in standard Plexiglas laboratory cages. The rats were provided with food and water *ad libitum*, and were maintained at 20°C, under a 12-h light/dark cycle (lights on 07:00–19:00 h), in a humidity-controlled environment. All experiments were started 1 week after the rats arrived, giving them an opportunity to acclimatize to their new environment. All experimenter interactions with the animals are done under aseptic conditions, with back-fastening gown and latex gloves worn at all times.

Animals were treated during the light phase of their circadian cycle. Depending on treatment, rats either received a once or twice (only in the case of lithium) daily intraperitoneal injection. Doses were based on weight, and all animals were weighed daily just before receiving the injection. Animals were handled by employing a crossover hold, where the experimenter applies upward motion on one of the scapulas with the thumb and downward motion of the opposite scapula with the index finger, causing the animals forelimbs to cross at the front of its neck. The body of the animal was cradled in the palm of the same hand. The animal was held in dorsal recumbency, with the head held slightly below the horizontal, enabling the abdominal viscera to shift cranially. It is imperative that the animals not move during the injection procedure to avoid an accidental damage to the viscera. During the injection, the needle was inserted at the midpoint of the lower right quadrant to avoid the cecum and urinary bladder. The needle was directed toward the animals head at an angle of about 20 degrees and inserted approximately 5 mm. The needle was aspirated to ensure that the abdominal viscera had not been penetrated. Following a clear aspiration, the plunger on the syringe was depressed and the treatment administered. Control animals were treated identically, and received a sham injection, of equal volume given to drug treated animals, of either saline or propylene glycol, depending on drug solubilities. Experiments ended either 12 hours (for twice daily treatments) or 24 hours (for once daily treatments) after the last scheduled injection.

The following list serves as a checklist of supplies – each of these items should be available and ready to use before beginning the treatment phase of the experiment:

Animal weigh scale
1 ml and 3 ml Luer-Lok syringes
27G$^{1}/_{2}$ precision glide needles
125 ml Erlenmeyer flasks
Freshly prepared solutions of drugs to be administered

C.3 Decapitation, Tissue Dissection, Homogenization and Extraction

Upon completion of treatment, animals were killed via decapitation. This was done in the room adjacent to the area where the animals were housed, to avoid unnecessarily stressing the remaining animals. Between each decapitation, the workspace was thoroughly cleaned. Immediately following decapitation, the brain was rapidly removed and immersed in 2-methylbutane kept on dry ice. This instantly froze the brain tissue and prevented cellular degradation and necrosis. Frozen brains were then removed, weighed, individual labeled and stored at -80 Celsius until dissection. Whole brains were dissected into specific regions according to stereotaxic demarcation. Dissection took place in a surgical suite, under aseptic conditions. Dissection was conducted on a filter paper covered Petri dish. The dish contained ice in an effort to slow the thawing process. New filter paper was put down at the beginning of each individual brain dissection. All brain dissections were carried out on the same day and by the same experimenter (BMM). Immediately following each brain dissection, dissected brain regions were labeled, weighed and kept at -80 Celsius until homogenization.

Following dissection brain regions were individual homogenized and metabolites of interest were extracted using an adaptation of the methanol/chloroform total lipid extraction proposed by Bligh and Dyer. This approach involves the serial addition of methanol, chloroform and distilled water to the sample with successive homogenizations, in a ratio of 2:1:1, respectively. This process must be carried out on a volume-by-weight basis, to control for variations in the amount of tissue dissected from each brain region. However, this will not control for the stereotaxic accuracy of the dissection. Following homogenization, a standardized volume of homogenate [based on the smallest (lightest) sample prepared] was transferred to an Eppendorf tube and centrifuged, resulting in a biphasic system (chloroform and methanol). For the present set of experiments it was desirable to remove the lipids, as they are NMR visible, forming broad peaks in the range of 0.9 – 1.2 ppm, primarily contributable to the methyl and methylene groups. In the biphasic system, the lipids are contained in the chloroform layer, and thus a standardized volume of the supernatant (methanol layer – containing metabolites of interest) was drawn up and transferred to a culture tube and kept at -20°C overnight, leaving behind the lipid-containing layer and tissue residue. The collected supernatant should be clear and colorless. If this is not the case, it may be filtered or centrifuged at a higher rpm. The following day, the supernatant was taken to dryness using vacuum centrifugation. After drying, residual methanol may remain, and would be detectable as a triplet peak centered at 3.15 ppm during NMR analysis. From this author's experience, a small amount of methanol usually remains; however, it has little or no overlap with brain metabolites of interest, and does not interfere with metabolite identification and quantification. The dried extract can be stored for prolonged periods at -80 Celsius or until preparation for NMR preparation, analysis and spectral quantification, but successive freezing and thawing should be avoided.

The following list serves as a checklist of supplies – each of these items should be available and ready to use before beginning the decapitation, tissue dissection, homogenization and extraction phase of the experiment:

Bench top centrifuge
Brain dissection atlas
Container of Ice
Cryovial rack
Freezer bags
Germex
Homogenization tubes
Kimwipes
Latex gloves
Mechanical tissue homogenizer
Micro-centrifuge tube rack
Nitrile gloves
Pestle
Pipette bulb
Small weigh boats
Vacuum Centrifuge

1 pair of surgical scissors
1 scalpel with #10 blade
1 pair of pliers
1 micro spatula
1 digit bench weigh scale
1 small Guillotine
1.2 ml exterior threaded cryovial
2 ml polypropylene micro-centrifuge tubes
9 cm diameter Petri dish
9 cm diameter coarse filter paper
12 mm x 75 mm Simport culture tubes
15 cm disposable transfer pipettes
50 ml ice-cold 2-methylbutane
50 ml beaker of 100% anhydrous ethanol
50 ml beaker of chloroform
100 ml distilled water

1 pair of 0.07 mm x 0.04 mm Dumont #7 biologie tip forceps

C.4 NMR Preparation, Analysis and Spectral Quantification

At the time of NMR analysis, the dried extract was reconstituted in a combination of distilled and deuterated water. An internal chemical shift reference standard of known concentration was also added to enable absolute quantification. As with the supernatant, the reconstituted extract should be clear and colorless, containing little or no brain residue. This is important, as large particulate matter will make shimming (discussed below) more difficult and may affect the quality of the data. The volume of reconstituted extract is also important, as the length of the receiver coil in most spectrometers typically range from 16-18 mm, meaning a sample length of about 42 mm is recommended to remove end effects. End effects result from the change in sample state at the interface of liquid to air. With the use of a 5 mm diameter NMR sample tube, this length (i.e. 42 mm) translates into an ideal sample volume of between 600-700 μL. Making the volume larger unnecessarily dilutes the sample, while a concentrated sample of lesser volume is more difficult to shim. In the present set of experiments, 0.6 ml of distilled and 0.06 ml of deuterated water (which contained 5 mM 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference standard, 100 mM imidazole and 0.2 % NaN_3) was added to the dry sample, at a pH of approximately 7. Since the pH of the sample can alter the chemical shift of the constituents, all samples should have similar pH, with 7 being the most common benchmark. Also, the physiological pH of 7 is particularly useful and relevant when studying drug action and brain neurochemistry. The reconstituted samples were transferred with a pipette into NMR tubes for analysis.

Spectroscopic analysis was carried out with an 18.8T Varian Inova-800 spectrometer. Samples were placed in the spectrometer, allowed to equilibrate and then shimmed before data acquisition. Shimming is a mechanical means of compensating for slight imperfections in the uniformity of the spectrometer magnetic field by inducing smaller magnetic fields inside the magnet core. Particulate matter in the sample (discussed above) as well as imperfections in the NMR tube itself can make this process less successful. Besides shimming, data quality can be improved by ensuring a high signal-to-noise. A higher signal-to-noise ratio can be achieved by repeating the data acquisitions and then summing the resulting signals. This is based on the premise that real signal for the sample will add, while random error will average out.

Once acquired, spectra were analyzed and metabolites were quantified using Chenomx NMRSuite Profiler software (v.4.0). This software package contains a compound library of over 200 unique spectral signatures that can be overlayed onto the acquired rat brain NMR spectra based upon the compound peak patterns. The addition of a known amount of the internal standard DSS enables the absolute concentrations of metabolites at μM concentration and above to be determined. Moreover, having the internal standard present in the mixture during spectral acquisition negates the prospect of needing to calibrate the instrument as is commonly observed for other analytical techniques such as mass spectroscopy or gas chromatography. In the present context, the determination of the concentration of the metabolites simply involves the multiplication of the area under the peak cluster by the number of protons resonating at that frequency divided by the area of the reference compound that has been multiplied by the number of protons resonating at its frequency. This computation is done by the Profiler software. The calculated concentration values are then exported to a statistical software package, where differences between treatment groups are analyzed.

The following list serves as a checklist of supplies – each of these items should be available and ready to use before beginning the NMR preparation, analysis and spectral quantification phase of the experiment:

Chenomx NMRSuite Profiler software
Culture tube rack
NMR tube caps
NMR tube rack

Personal Computer with statistical software package
8.7 mm x 38 mm NMR tube labels
15 cm disposable transfer pipettes
18.8 Tesla Varian Inova-800 spectrometer and console
50 ml of deuterated water (containing 5mM DSS)
50 ml of distilled water
178 mm round bottom NMR tubes

Appendix D. Conference Presentations

10. Weljiel AM, McGrath BM, Taylor J, Silverstone PH, Jirik FR, Vogel HJ, Newton J. Targeted Profiling of Metabolomic Data for Predictive and Pathway-Specfic Animal Models. Metabolomics 2006: 2nd Scientific Meeting of the Metabolomics Society. Boston, MA, June 2006.

9. McGrath BM, Hanstock CC, Seres P, Dave S, Silverstone PH. Phosphinositol Metabolism in Depression: Is there a difference between Bipolar and Unipolar Disorders? 61st Annual Meeting of the Society of Biological Psychiatry. Theme: Vulnerability and Resilience – Implications for Psychiatric Disorders. Toronto, Canada, May 2006. *Biological Psychiatry*, 59(8, Suppl 1):136S, 2006.

8. McGrath BM, Greenshaw AJ, McKay R, Hanstock CC, Seres P, Slupsky CM, Weljie AM, Dave S, Silverstone PH. Dextro-Amphetamine is not a PI-cycle based Model of Mania: Data from Clinical (3T) and Preclinical (18.8T) MRS studies. 61st Annual Meeting of the Society of Biological Psychiatry. Theme: Vulnerability and Resilience – Implications for Psychiatric Disorders. Toronto, Canada, May 2006. *Biological Psychiatry*, 59(8, Suppl 1):52S, 2006.

7. Tibbo P & McGrath BM. University Students' Perceptions of the Stigma toward Mental Health Consumers and their Families: Does Perception of Stigma influence Willingness to Seek Help for Mental Health Problems? 55th Annual Meeting of the Canadian Psychiatric Association. Theme: Building Networks, Crafting Excellence. Vancouver, Canada, November 2005.

6. McGrath BM & Tibbo P. Perceptions of Stigma and Willingness to Seek Mental Healthcare among Canadian University Students. Alberta Mental Health Board. Mental Health Research Showcase. Theme: Advancing Mental Health Through Research, Innovation and Knowledge Translation. Banff, Canada, November 2005.

5. McGrath BM, Greenshaw AJ, McKay R, Hanstock CC, Seres P, Weljie AM, Slupsky CM, Dave S, Silverstone PH. Clinical and Preclinical Effects of Acute Dextro-Amphetamine on Phosphoinositol Metabolism in Brain. The International Society for Magnetic Resonance in Medicine (ISMRM). Theme: MR Spectroscopy for Neuropsychiatric Disorders. Banff, Canada, October 2005.

4. McGrath BM, Hanstock CC, Dave S, Seres P, Silverstone PH. Phosphinositol Metabolism in Patients with Bipolar and Unipolar Depression. The International Society for Magnetic Resonance in Medicine (ISMRM). Theme: MR Spectroscopy for Neuropsychiatric Disorders. Banff, Canada, October 2005.

3. McGrath BM, Greenshaw AJ, McKay R, Hanstock CC, Dave S, Silverstone PH. Acute Dextro-Amphetamine Administration does not alter Phosphoinositol Metabolism in the Brains of either Humans or Animals. 18th Congress of the European College of Neuropsychopharmacology. Amsterdam, The Netherlands, October 2005. *European Neuropsychopharmacology*, 2005.

2. Tibbo P & McGrath BM. Does Progression Through the Medical Curriculum Influence Students' Perceptions of Mental Illness Stigma and their Willingness to Seek Help for Mental Health Problems: A Naturalistic Study? Annual Meeting of the Association for Academic Psychiatry. Theme: Maximizing our Academic Capital: New Inroads from the Fields of Business, Social Anthropology, and Technology. Chicago, USA, September 2005.

1. McGrath B.M. Toward a Comprehensive Understanding of Bipolar Neuropathophysiology: NMR Spectroscopic Investigation of In Vivo Neurochemistry and Ex Vivo Drug Action. The 3rd Annual CIHR – INMHA Meeting: A Feast of Science and Partnership. Ottawa, Canada, November 2004.